EFFECTS OF EXPERIMENTAL JAUNDICE ON ADRENAL CORTICAL ACTIVITY IN RATS

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By

William P. Baker

AN ABSTRACT

Submitted to the School of Graduate Studies of
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DOCTOR OF PHILOSOPHY

rmacology

ABSTRACT

1. Experimental jaundice was produced in rats by ligating the common bile duct. The effects of the resulting jaundice on adrenal cortical activity, adrenal function and liver inactivation of hydrocortisone (compound "F") were studied.
2. Ligation of the common bile duct of rats resulted in a marked increase in the icteric index of the plasma. The average icteric index reached a maximum of 47 on the fifth post-operative day and then declined slowly due to the recovery of some of the animals. The icteric index was found to average only 3.9 in normal control rats. Gross and microscopic changes typical of obstructive jaundice were seen in the rats made jaundiced by this procedure.
3. Twenty-four male rats were used in an experiment to determine the effects of experimental jaundice on adrenal cortical activity. On the eight day following bile duct ligation the animals were autopsied and it was found that the thymus of the jaundiced rats had undergone marked involution. This effect could not be attributed to the surgery or reduced food consumption since a sham-operated, pair-fed group of rats failed to show thymic involution. It was noted however, that jaundice was accompanied by decreases in food consumption and body weight. There was no significant difference in the weight of the adrenals between the jaundiced and non-jaundiced rats.

4. In another experiment, thirty rats were made jaundiced and were sacrificed on the sixth post-operative day. Three 4.5-6.0 mg. cotton pellets were implanted subcutaneously in each of the rats at the time of bile duct ligation or sham-operation. These pellets plus the granuloma formed around the cotton were removed and weighed at the time of sacrifice. In addition, the thymus, liver, and adrenals were weighed and the adrenal ascorbic acid was determined. Reduced granuloma formation and a decrease in thymus weight were found in the jaundiced rats but not in the sham-operated, pair-fed controls. There was increased liver weight in the icteric rats but no significant difference occured between the adrenals ascorbic acid levels of the jaundiced and non-jaundiced animals.
5. Thirty male rats were used in another experiment to determine whether or not a functional adrenal cortex was required for the previously observed effects of jaundice on thymus weight and granuloma formation. Some of the rats which were both adrenalectomized and jaundiced succumbed, making the data somewhat difficult to interpret accurately. It was observed however, that thymus involution and granuloma inhibition did not occur in the jaundiced rats which were adrenalectomized. This suggests that a functional adrenal cortex is required for the increase in adrenal cortical activity seen in experimental jaundice in rats.
6. The effects of experimental jaundice on the survival of

adrenalectomized rats given a single dose of compound "F" was studied in forty mature rats. These rats were given a large dose of compound "F" just prior to adrenalectomy. In addition, the common bile ducts of some of the animals were ligated. It was observed that the jaundiced adrenalectomized rats given compound "F" did not survive as long as the similarly treated non-jaundiced animals. It was concluded that jaundice probably does not interfere with the inactivation or excretion of this adrenal steroid. An increased utilization of the adrenal steroids may be indicated.

7. An experiment was performed on sixteen rats to determine whether or not experimental jaundice affects the <u>in vitro</u> inactivation of hydrocortisone (compound "F") by surviving liver slices. Eight of these rats were made icteric by ligation of the common bile duct. The remaining eight animals were sham-operated and pair-fed to the jaundiced group. On the sixth post-operative day the rats were sacrificed, the livers removed, and liver slices were prepared with a razor blade. Slices from each of the sixteen livers were incubated for three hours in Ringers' solution containing a known quantity of compound "F". Following this incubation period, the remaining compound "F" was extracted and determined chemically (by paper chromatography) and by means of a biological assay based on thymic involution. The chemical analysis showed that icteric and non-icteric liver slices inactivated similar quantities of compound "F". The biological assay showed

that a large quantity of compound "F" was inactivated by both the jaundiced and non-jaundiced liver and that the effects of both on thymus weight were similarly negative. It was concluded that experimental jaundice does not result in decreased inactivation of this adrenal cortical hormone.

8. It is suggested that thymic involution and reduced granuloma formation in rats and perhaps the disappearance of arthritic symptoms in jaundiced human patients are brought about by increased sensivity of the body tissues to adrenal steroids or to other mechanisms not yet determined.

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INTRODUCTION

Many investigations have been carried out recently in an attempt to gain a better understanding of rheumatoid arthritis and to develop a better means of combating this and related diseases. Among the results of these studies were two types of clinical evidence which have led to the present experiments on the effects of jaundice on adrenal cortical function and corticosteroid inactivation. First was the observation of Hench (1938a) that "when patients with rheumatoid (atrophic, chronic infectious) arthritis or primary fibrositis become definitely jaundiced a notable event usually occurs: their rheumatic symptoms are rapidly, markedly and generally completely alleviated for some weeks or months". Second among the clinical findings was the report of Hench et al. (1949) that the administration of large doses of cortisone or ACTH to patients with rheumatoid arthritis resulted in dramatic and prompt relief of the arthritic symptoms. While the above has definitely been established, there is as yet little factual evidence of any definite relation between jaundice and adrenal cortical function.

With the above observations in mind it became of interest to attempt to determine whether or not (a) experimental jaundice in rats is accompanied by increased adrenal cortical activity and (b) if so, whether the increased activity can be attributed to increased adrenal cortical function, reduced adrenal corticoid inactivation by the liver or to other mechanisms.

REVIEW OF LITERATURE

Jaundice and Arthritis. Although Hench (1933a) was the first to publish detailed case reports of the beneficial effects of jaundice on arthritic symptoms, this phenomenon had been mentioned previously by Still (1897), Wishart (1903) and in three reports on cinchophen toxicity by Parsons and Harding (1932a, 1932b) and Grigg and Jacobsen (1933). Cinchophen is a compound which was often used prior to 1935 for combating the pains of arthritis. The use of this substance often resulted in acute yellow atrophy of the liver and severe jaundice. These toxic effects have greatly reduced its value and use as an analgesic agent. Since the first report of Hench (1933a), other workers including Sidel and Abrams (1934) and Borman (1936) have reported similar observations concerning the beneficial effects of jaundice in arthritic patients. Hench has remained the most active in the study of this phenomenon as evidenced by his further reports (1933b, 1934, 1935, 1937, 1938a, 1938b, 1938c).

All of the above reports agree that symptomatic relief is seen in a large percentage of arthritic patients when they become jaundiced. The nature of the relief is a notable or complete remission of articular pain, articular swelling, muscular stiffness, soreness, and fatigue. The alleviation of arthritic symptoms is prompt with the occurance of jaundice and the relief persists an average of three times the duration of the icterus. Great variation is seen, however, in duration of

the relief of arthritic symptoms (Hench 1938a). Hench (1938a) stated that the effectiveness of jaundice is more dependent on the severity of the icterus than on the type. Also the minimum severity of icterus required for relief from arthritis corresponds to approximately 8 mg. of bilirubin per 100 cc. of plasma.

Hench (1938a) postulated that the substance responsible for the disappearance of arthritic symptoms in jaundiced individuals may be a constituent of bile such as bilirubin bile salts, mucin, a lipid, or perhaps an abnormal hepatic substance such as hepatic autolase. He also included in this list of possibilities a "Substance X" of extra hepatic origin. He stated that the same substance was probably responsible for the relief of arthritis in jaundice as in pregnancy, and that it was probably not bilirubin since tissue bilirubin is low in pregnancy, Also the analgesia persists after the bilirubin of jaundice has returned to normal.

Pemberton (1920) postulated on the basis of remission of arthritic symptoms seen in a case of catarrhal jaundice that the effect was due to low dietary intake. Hench (1938a) reduced the dietary intake of arthritic patients and observed no improvement. He also stated in the same report that this phenomenon is not a case of simple sedation because pains of other origin are not alleviated by jaundice, and also the swelling of the affected joints is visibly reduced as a result of icterus.

Hench (1938a) has made many attempts to reproduce the therapeutic effect of jaundice and thus determine the factor responsible for this relief of arthritic patients. Hyperbilirubinaemia induced by administering bilirubin intravenously produced slight alleviation in these cases but the relief was much less pronounced than seen in similar bilirubin levels resulting from spontaneous jaundice. Massive oral doses of ox bile in combination with intravenous bile salt also gave questionable results. Transfusions of arthritic patients with highly jaundiced blood was only transiently effective in one of several patients treated in this manner. The question of which factor is responsible for the remission of arthritic symptoms in jaundiced individuals remains unanswered. However, it would appear from the reports just cited that this substance is not one of the well known constituents of normal bile.

The Systemic Effects of Jaundice. Jaundice is a symptom of many liver diseases and extra hepatic disorders. Hepatitis, bile duct obstruction, hemolysis, and liver damage by various toxic agents are amoung the most common causes of this condition. For an exact classification and discription of various types of jaundice the interested reader is referred to the discussion of Schiff (1948).

Inasmuch as icterus was produced in this study by ligating the common bile duct, only the effects of experimental obstructive jaundice will be considered here. This material is taken almost entirely from the book written by Horrall (1938). Accor-

ding to this author, ligation of the common bile duct of dogs results in an increased circulating level of bilirubin, cholesterol and the bile salts. The resulting experiental jaundice is accompanied by cirrhosis of the liver, ascites, cachexia, slowing of the heart, a decline in blood pressure and general intoxication. Horrall (1938) also stated that in experimental obstructive jaundice a condition may develop which resembles that seen in hepatectomized animals. Blood glucose may decrease while the level of uric acid increases. At this stage of liver function impairment, amino acids are not metabolized and the circulating level of prothrombin is greatly reduced. As a result such animals have a greatly increased clotting time.

The absence of bile from the intestinal tract as a result of obstructive jaundice results in decreased intestinal motility and impaired absorption of fat, fat soluble vitamins and calcium (Horrall 1938). Thus nutritional deficiencies often develop in addition to the other changes resulting from bile duct obstruction. Dogs live from 40 to 65 days following ligation of the common bile duct while rats live only 23 days. Whether or not the death of these animals is a result of a toxic factor in bile or hepatic insufficiency is difficult to ascertain. Horrall (1938) concluded however, that the toxic effects which are attributable to bile are due to the bile salts and not to bile pigments. He stated that bile pigments are practically inert. Thus bile salt levels would

be more meaningful than the icteric index if such values were easily obtainable.

The Adrenal Cortex and Jaundice. Since the 11-oxy adrenal steroids and ACTH have been the only chemotherapeutic agents to approach the therapeutic effectiveness of jaundice and pregnancy in arthritic patients, the question arises as to whether this beneficial effect of jaundice is due to an elevated circulating level of adrenal steroids. Also, if the blood level of corticosteroids is elevated in jaundiced patients, is this accomplished by increased adrenal cortical function or by a reduced steroid inactivation? There is indirect evidence which suggests that both phenomena may occur in jaundiced individuals. Present data are insufficient however to prove unequivocally either of the above mentioned hypothesis.

Selye (1950) has shown that almost any specific or non-specific stress contributes to an increased secretion of adrenal steroids. The meaning of the term "stress" as used by Selye has been extended to include almost every known metabolic and infectious disease. Thus it would not be surprising if the diseases which contribute to jaundice, or even jaundice itself could also be included in the already long list of stressor agents. Godfrey (1947) reported his observation of hemorrhage in the adrenals of a patient suffering from severe jaundice. This condition of adrenal hemorrhage had been shown by Selye (1950) to result from many diseases

and has been attributed to systemic stress by this author.

Urinary Steroids in Liver Disease. Whether or not the catabolism of adrenal steroids is impaired in liver disease is a point of conjecture. The solution of this problem is difficult because of the uncertainity involved in relating the steroid metabolites of the urine to their precursors. Lieberman and Teich (1953) stated that more than 100 steroid substances have been isolated from urine. These authors stated further that it is impossible on the basis of present knowledge to eliminate any of the non-benzenoid compounds as not being formed from adrenal steroids. Samuels and West (1952) conclude that approximately twenty-seven of these urinary steroids arise from adrenal steroid precursors. These authors have shown that some of these 27 compounds are obtainable only from persons with adrenal cortical tumors and therefore may not represent a natural pathway of steroid metabolism.

Unfortunately few authors have used adrenal steroids in studies on steroid metabolism. Instead estrogens and androgens have been most thoroughly studied in this respect. Inasmuch as all steroids are metabolized somewhat similarly, the information gained from studies of one steroid are often beneficial in understanding the metabolism of another. Thus many of the in vivo reactions of the androgens and adrenal steroids are identical after the first few steps of their enzymatic degradation. Both of these types of hormones ultimately contribute to the 17-ketosteroid titer of the urine. There are certain

specific differences in the metabolic pathways of various steroids and variations in the manner in which different species metabolize the same steroid substance. These specific differences cannot be described here. The interested reader is referred to the review of Lieberman and Teich (1953).

The urinary steroids occur primarily as conjugates of sulfuric and glucuronic acid. The percentage of the given steroid occuring in the urine as a conjugate depends on the chemical nature of the steroid as well as the nutritional status of the animal and condition of the liver. While most of the cortisone and hydrocortisone of normal urine is in the free state, Venning et al. (1953) and Katzman et al. (1952) reported that the major portion of the urinary 17-ketosteroid titer is in the form of glucuronides.

Cantarow et al. (1951) found larger percentages of free 17-ketosteroids, as compared to conjugates, in the urine of patients with liver disease than in normal persons. Bogiovanni, and Eisenmenger (1951) also found considerably smaller fractions of ketosteroids excreted as glucuronides in cirrhotics as compared to normal individuals. These authors conclude that hepatic damage impairs the process of steroid conjugation.

Samuels and West (1952) compared the renal clearances of free steroids and their congugated forms with creatinine. From their results they concluded that the ease with which a given steroid can become conjugated with either glucuronic or sulfuric acid determines which steroid substances will appear

in the urine. The steroid conjugates were freely filtered at the glomerulus and removed from the metabolic pool while the free steroids complexed with the serum proteins, particularly the albumins, and remained in the circulating plasma.

Liver function and nutrition are not only important in the conjugation and subsequent urinary excretion of steroid substances, but are also primarily involved in the catabolism of these endrocrine products to an inactive form. Glass et al. (1940, 1944) have demonstrated feminization, particularly gynecomastia and testicular atrophy, in male patients with cirrhosis of the liver. This feminization was accompanied by an increased urinary excretion of free estrogens. Gilder and Hoagland (1946) have reported similar findings in eleven male patients with acute infectious hepatitis. Wu (1942) has shown typical estrogenic stimulation of the prostate in male patients with liver damage. In female patients with cirrhosis, Biskind and Biskind (1942) have found evidence of excessive estrogen stimulation of the female genital tract. Dohan et al. (1952) studied the biliary excretion of estrogens in persons with hepatic disease and concluded that the increased urinary excretion of estrogens in persons with liver disease is a result of reduced biliary excretion of these substances.

Paschkis et al. (1951) found that the excretion of pregnandiol after administration of a standard dose of progesterone is higher in persons with liver disease than in normal subjects. These authors concluded that the breakdown of progesterone to

pregnandiol is not impaired by liver damage but that further metabolism of this intermediary substance is reduced.

Relatively little work has been done on steroid excretion in connection with adrenal steroid elemination in liver disease. Sprague et al. (1951) studied the urinary corticoid excretion of 17 patients with various liver diseases such as obstructive jaundice, cirrhosis and viral hepatitis. With the exception of one case in which the urinary corticoid level was low, the urinary excretion of corticoids by the sixteen remaining patients was within the range of normal. In this same study two jaundiced patients were given therapeutic doses of cortisone daily. The 17-ketosteroid excretion decreased from a subnormal level to an even lower level as a result of cortisone administration, while the corticoid excretion increased six fold.

These authors concluded that an increased adrenal function sufficient to alleviate the symptoms of rheumotoid arthritis should be reflected in an increased urinary excretion of corticosteroids but stated that their data were too few to permit definite conclusions. The decrease in 17-ketosteroid excretion in jaundiced individuals following cortisone administration has been observed repeatedly in subjects with normal liver function by the same authors. They attribute this decline in 17-ketosteroids excretion to a supression of adrenal cortical function. In such cases it is believed by Sprague et al. (1951) that the decrease in the amount of endogenous 17-ketosteroids is greater than the amount of 17-ketosteroids formed

by metabolism of the administered cortisone suppress the excretion of other steroids substance which are more easily converted to 17-ketosteroids than is cortisone itself. Eymer and Moll (1953) also observed a reduced 17-ketosteroid excretion in persons with liver disease. There was only an insignificant rise in the excretion of these steroids following ACTH administration. The corticoid excretion was normal in these individuals and reacted normally to ACTH stimulation. Thus it would appear from the data of Eymer and Moll (1953) that while the pathways of degradation of corticosteroids to 17-ketosteroids are impaired in liver disease, those leading to corticoids are not altered.

Butt et al. (1951) treated two patients, one with hepatitis and the other with biliary cirrhosis, with cortisone acetate. This therapy did not affect either the results of liver function tests or the lipemia which accompanied these diseases. These authors also reported that no more than the usual amount of cortisone escaped metabolism but that smaller amounts of compound "E" were converted into 17-ketosteroids in patients with liver disease. The implication is that while the conversion of cortisone to 17-ketosteroids is impaired, other routes of metabolism are sufficient to prevent a reduced inactivation of cortisone in patients with cirrhosis or hepatitis. However the fact that only two patients were used in these studies limits the conclusiveness of the data.

Lieberman and Teich (1953) have analysed much of the

data pertaining to the urinary excretion of steroid substances in normal persons and patients with liver disease. From these data they were able to arrive at the following conclusions: the reactions involved in the conversion of estrogens to their unidentified metabolic products are impaired in patients with liver damage. Thus elevated urinary excretion of free estrogens and increased estrogenic stimulation is often seen in these patients. The ability of the liver to convert the 17-hydroxyl group of testosterone to a 17-ketone function is also impaired in liver disease, the result being a reduced 17-ketosteroid excretion. A diseased liver is also less capable of conjugating steroid substances into forms more easily excreted. This is especially true in connection with glucuronide formation. Finally these authors conclude that the ability of the liver to degrade the side chain of carbon 17 of the adrenal steroids may be reduced in liver disease. This phenomenon may also contribute to the subnormal 17-ketosteroid excretion in patients with hepatitis or cirrhosis.

Nutrition and Steroid Excretion. The role of nutrition in steroid inactivation has been most widely studied in the case of estrogens. Biskind (1946) has observed in some 450 patients "a striking correlation between the signs and symptoms of nutritional deficiency and the occurance of syndromes related to excess estrogen". This same author has shown that in male and female rats the ability of the liver to inactivate estrogens was greatly reduced when the animals were fed a vitamin B

complex free diet. This reduced estrogen inactivation occured as early as two or three days after the rats were placed on the deficient ration. There were no detectable morphological changes in the livers of these animals. Shipley and Gyorgy (1944) have reported similar findings of excessive estrogenic stimulation in rats following a vitamin B complex deficiency.

Relatively little work has been done which is concerned directly with nutritional deficiencies and adrenal steroid metabolism. Meites (1951) showed that a vitamin B_{12} deficiency aggravated the inhibitory effects of large doses of cortisone on body, hair and thymus growth in rats. Meites (1952) later repeated this work with another diet and reported similar findings. He suggested that the effect of vitamin B_{12} in preventing or reducing these manifestations of cortisone may be due to an essential role of this vitamin in protein metabolism. Thus the nitrogen sparing effects of vitamin B_{12} partially or completely offset the negative nitrogen balance produced by large doses of cortisone (Meites and Feng, 1953).

Wahlstrom and Johnson (1951) have observed an increased urinary excretion of vitamin B_{12} in baby pigs following cortisone administration and Lang et al. (1953) made similar observations in rats. In view of the evidence cited earlier with respect to nutrition and estrogen inactivation, it would not seem unreasonable to attribute the increased effectiveness of cortisone in vitamin B_{12} deficiency at least in part to a decreased rate of cortisone inactivation. Evidence to be

presented later in connection with *in vitro* studies on steroid inactivation make this hypothesis even more tenable.

Landau et al. (1948) demonstrated a fifty percent reduction in the urinary 17-ketosteroid excretion in three persons following three days of starvation. To what extent this result is attributable to diminished androgen secretion or to reduced steroid catabolism is not known.

Steroid Excretion in Bile and Feces. The availability of radioactive labeled steroids has made possible many recent studies of biliary and fecal excretion of steroid substances. The fact that the bile ducts were ligated in many of the animals used in this thesis problem makes at least a portion of this material pertinent.

Twombly (1951) observed that a short time after the intravenous injection of labeled dibromoestrone in rabbits, high concentrations of radioactivity appeared in the gall bladder. This activity was later found in the feces and intestinal contents. This same author injected labeled dibromoestrone intravenously in bile festula dogs and was able to isolate seventy percent of the injected activity in the bile during the first five hours. During this same time period only four percent of the injected activity appeared in the urine. Twombly (1951) found similar patterns of excretion of this labeled estrogen in dogs, rats, and mice as well as in humans. It is of interest that persons with the least evidence of liver disease excreted the highest percentage of the injected activity in bile.

Barry et al. (1952) reported that the greatest portion of the activity resulting from the administration of C^{14} labeled testosterone as well as C^{14} labeled progesterone occured in the feces in rats and also in mice. These authors postulate that the major portion of the radioactivity resulting from the injection of these two steroid hormones is excreted into the bile and thence into the feces.

At present, isotopically labeled adrenal steroids have not been available in sufficient quantities and for sufficient periods of time to make possible detailed studies of their excretory patterns. Lieberman and Teich (1953) stated that "no steroid has been isolated from bile which can be related unequiocally to the adrenalcorticoids". However they stated further that some of the steroids present in bile may conceivably be products of adrenal steroid catabolism. Studies using radioactive labeled adrenal steroids would offer the most obvious solution to this problem. Such studies are undoubtedly being conducted at this time.

Albert et al. (1949) found that ligation of the common bile duct in mice caused much higher levels of alpha-iodo-estradiol to be excreted in the urine. Twombly (1948) injected radioactive labeled dibromo-estrone into the gall bladder of rabbits and was able to demonstrate radioactivity later in the blood and urine. Nicholas et al. (1951) showed that more than one half of the radioactivity injected intravenously as labeled progesterone is excreted into bile of rats.

Ligation of the common bile duct greatly increased the urinary excretion of radioactivity in these animals.

Gallagher et al. (1951) studied the excretory pathways of isotopically labeled testosterone in rodents and in man. These authors were unable to detect any known neutral ketosteroid in the neutral ketonic fraction of human feces. In rabbits however, more than fifty percent of the intraperitoneally injected C^{14} labeled testosterone appeared in the feces within the first twenty-four hours after injection. High levels of radioactivity occured in the gall bladders of these animals soon after the injection of the labeled testosterone.

From the evidence cited here it is apparent that bile represents one of the major pathways of steroid excretion. Thus it can be said that any factor which affects the production or elimination of bile such as obstruction of the bile duct, cirrhosis of the liver, or hepatitis is likely to alter the pathways and rate by which steroids and their metabolites are eliminated. It was seen earlier that when the bile ducts of rats and mice were occluded, greater portions of steroid metabolites were excreted in the urine. In view of this it is not surprising that Twombly (1951) found that persons with the least evidence of liver disease excreted the highest percentage of radioactive estrogen into the bile.

Studies of In Vitro Steroid Inactivation. Inasmuch as liver has been shown to be the principal cite of inactivation

of almost every known steroid hormone, much work has been done using liver slices and liver perfusion techniques in an attempt to characterize the chemical reactions involved in steroid metabolism. These studies have been the subject of many reviews, one of the most recent being that of Lieberman and Teich (1953). While these studies are not entirely unrelated to this thesis problem, their great volume and complexity makes impractical any attempt by this author to comprehensively review this material. It can be pointed out however, that the reactions involved in the inactivation of the adrenal steroids fall largely into two classes. The first of these reactions is the reduction of the α, β, unsaturated carbonyl group in the A ring of the steroid nucleus. A second group of reactions degrades the dihydroxyacetone or hydroxy-acetone grouping affixed to carbon 17 of the adrenal steroids. Cortisone (E), hydrocortisone (F), 11-desoxycortisone (S), 11-desoxycorticosterone (DOC), corticosterone (B) and 11-dehydrocorticosterone (A) all exhibit life maintainance activity in adrenalectomized rats. Since all of these compounds possess the characteristic α, β, unsaturated carbonyl group in the A ring as well as a hydroxy-acetone group at carbon-17, it has been assumed that any reaction producing an alteration in either of these groups will result in inactivation of the adrenal steroid in question. Six of the biologically active adrenal steroids are shown in Fig. 1.

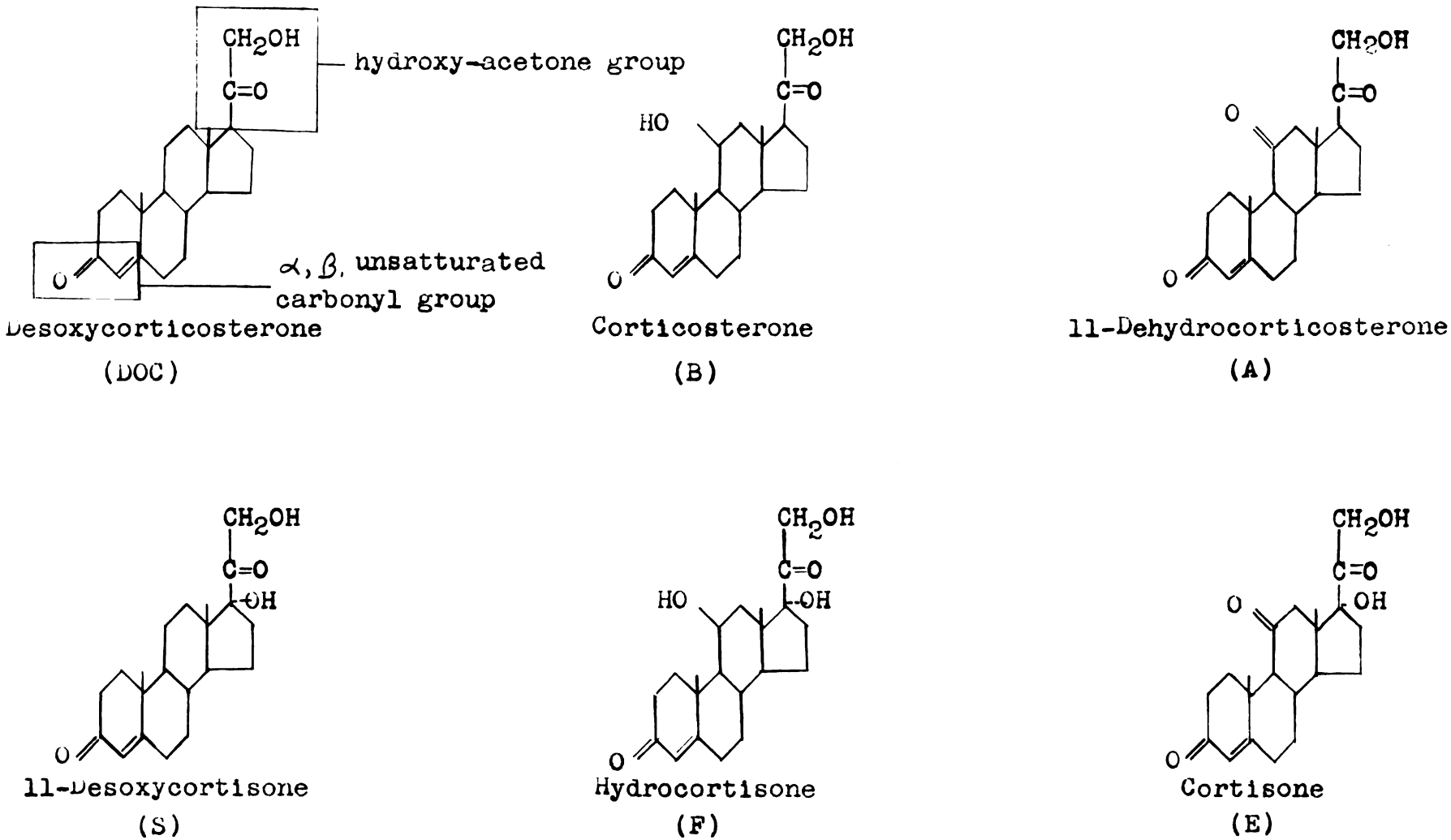

Fig.1. Six biologically active adrenal steroids showing the hydroxy-acetone and α, β, unsaturated carbonyl groups which are common to all six steroids.

Paschkis et al. (1951) incubated cortisone with various tissues and measured the extent of inactivation by the liver-glycogen method of Venning et al. (1946). These authors found that all tissues studied, including diaphragm and brain as well as liver, were equally effective in inactivating this adrenal steroid. Schneider and Horstmann (1951) incubated desoxycorticosterone with various surviving rat tissues. Chemical anaylsis of the system following three hours of incubation under an atmosphere of oxygen revealed that the conjugated unsaturated function of the A ring had been largely reduced. Surviving slices of kidney were capable of carrying out this same reaction but at a much reduced rate. Degradation of the hydroxy-acetone side chain of carbon-17 was also more rapid in the case of liver than with kidney.

Schneider and Horstmann (1952) later carried out similar studies in which they determined the rate of inactivation of cortisone and related adrenal steroids by several surviving rat tissues. Again liver was much superior to the other tissues studied in both the reduction of the A ring and in degrading the two carbon side chain of carbon-17. Compounds "A", "E" and "F" were most rapidly inactivated while "DOC" and compound "S" were significantly more resistant to the catabolic reactions studied. In these studies, 3.0 mg. of cortisone was inactivated in three hours by each gram of liver as measured by the disappearance of the conjugated unsaturated system. The loss of the 17,21-dihydroxy-20-keto structure

averaged 2.4 mg. of compound "E" per gram of rat liver in three hours. Kidney slices were approximately one third as active in the inactivation of cortisone while adrenal slices were only slightly active and muscle (diaphragm) showed no inactivation of this hormone at all. It is also of interest that guinea pig liver inactivated cortisone about one half as fast as did rat liver under similar conditions. It is also noteworthy that rat liver slices proved twice as effective as homogenates of the same tissue. Clark (1949) earlier incubated cortisone with liver slices and breis and found a similar rate of reduction of the α, β, unsaturated A ring.

Harding and Nelson (1951) determined the level of circulating 17-hydroxycorticoids in arterial and venous blood of different organs and tissues. These authors found no difference between arterial and venous levels in the case of kidney or muscle but reported a 30% drop in the concentration of these compounds between blood drawn from the arterial system and the hepatic vein.

It is apparent from the above studies that the liver is the principal site of adrenal steroid inactivation. Therefore diseases which tend to destroy or impair liver function may alter the rate of adrenal steroid inactivation. It is not clear however, how extensive liver disease must be in order to impair adrenal steroid metabolism. Thus it is uncertain what fraction of the total liver is required to inactivate

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the normal output of adrenal steroids. Also an estimation of the extent of hepatic damage in varying conditions and in different species may not be applicable to other conditions and to other species.

MATERIALS AND METHODS

Experimental Induction of Jaundice. Several experiments were required to develop a procedure for producing experimental jaundice in rats. In these experiments observations were made on the severity and duration of the jaundice as well as on gross and microscopic changes in certain tissues as a result of sustained icterus.

It was found that jaundice could be most readily induced by ligating the common bile duct in the region of its junction with the duodenum. A loop of duodenum was withdrawn through an incision made just caudal to the last rib on the right side of the rat. The bile duct was clearly visible in the mesentry in the region of the pancreas. The common bile duct was then ligated and the loop of duodenum inserted back into the peritoneal cavity. The incision was then closed by a suture in the body wall and a wound clip in the skin. All surgery was done under ether anesthesia. The point at which the common bile duct was ligated in this procedure is shown in Fig. 2.

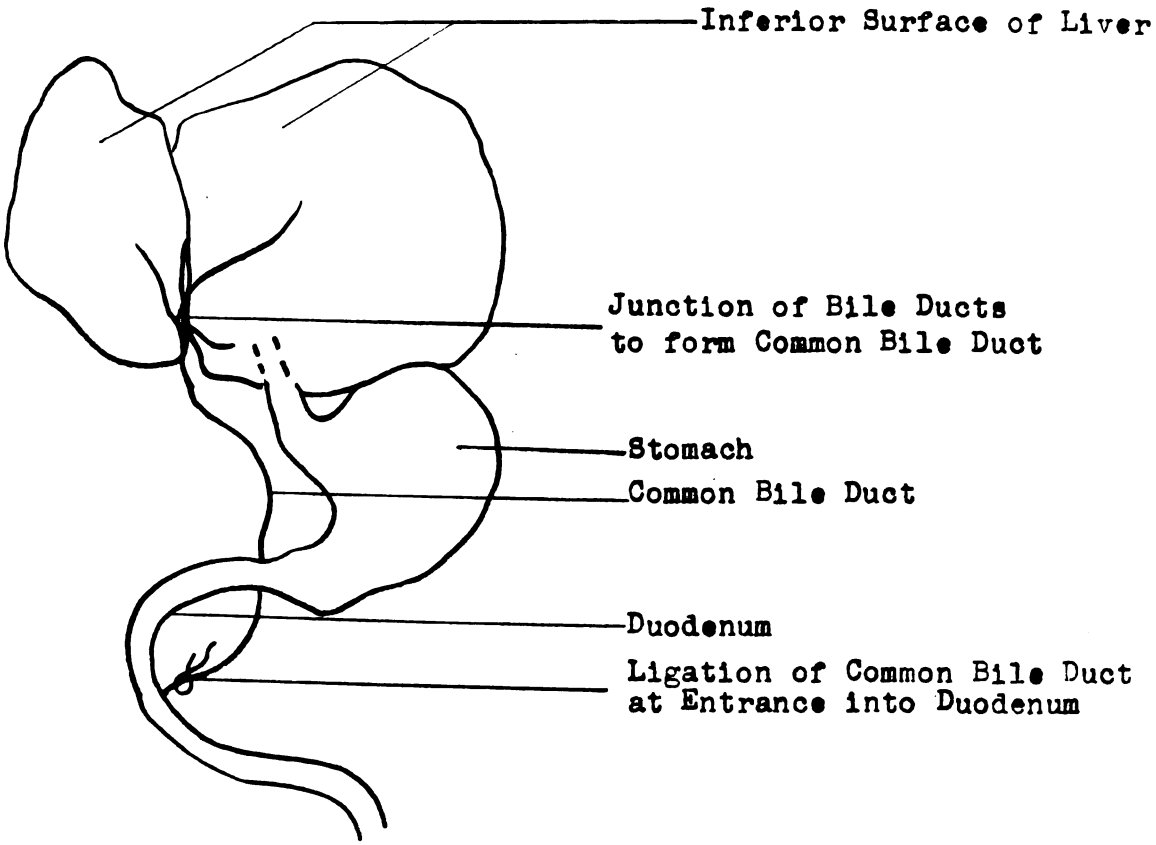

Fig.2. Sketch showing the point where the common bile duct was ligated in producing experimental jaundice.

At different time intervals following this operation rats were anesthetized, a laparotomy was performed and blood was withdrawn from the abdominal aorta into a heparinized syringe. These blood specimens were then centrifuged and a sample of plasma was removed for icteric index determinations. The icteric index was determined according to a standard procedure described by Hawk et al. (1951). Diluted plasma was compared to a standard potassium dichromate solution in a Fischer colorimeter using a 425A filter. The icteric index of normal rat plasma was found to be approximately 3.9 as determined by this procedure. Hawk et al. (1951) stated that normal human plasma has an icteric index of 4 to 7 and that icteric symptoms are observed above 15.

Table I shows the icteric index of plasma withdrawn from rats at various time intervals following ligation of the common bile duct. These data is presented graphically in Fig. 3.

It is apparent that the icterus increases to a maximum approximately 5 days following the ligation of the bile duct. After this time the mean icteric index decreases due to the recovery of some of the rats. The reason for this recovery is not clear.

Table I. Mean Icteric Index of Rat Plasma at Different Time Intervals Following Ligation of the Common Bile Duct.

No. of Rats	Time Lapse Following Bile Duct Ligation	Mean Icteric Index
5	12 hours	19.8±1.5*
1	24 hours	23.0
6	48 hours	44.2±2.4
4	60 hours	32.5±7.8
5	3 days	37.2±3.3
7	5 days	47.0±6.5
15	7 days	41.4±5.9
8	8 days	33.8±13.7
3	13 days	7.6±4.7
16	23 days	14.4±5.3

*Standard error of mean

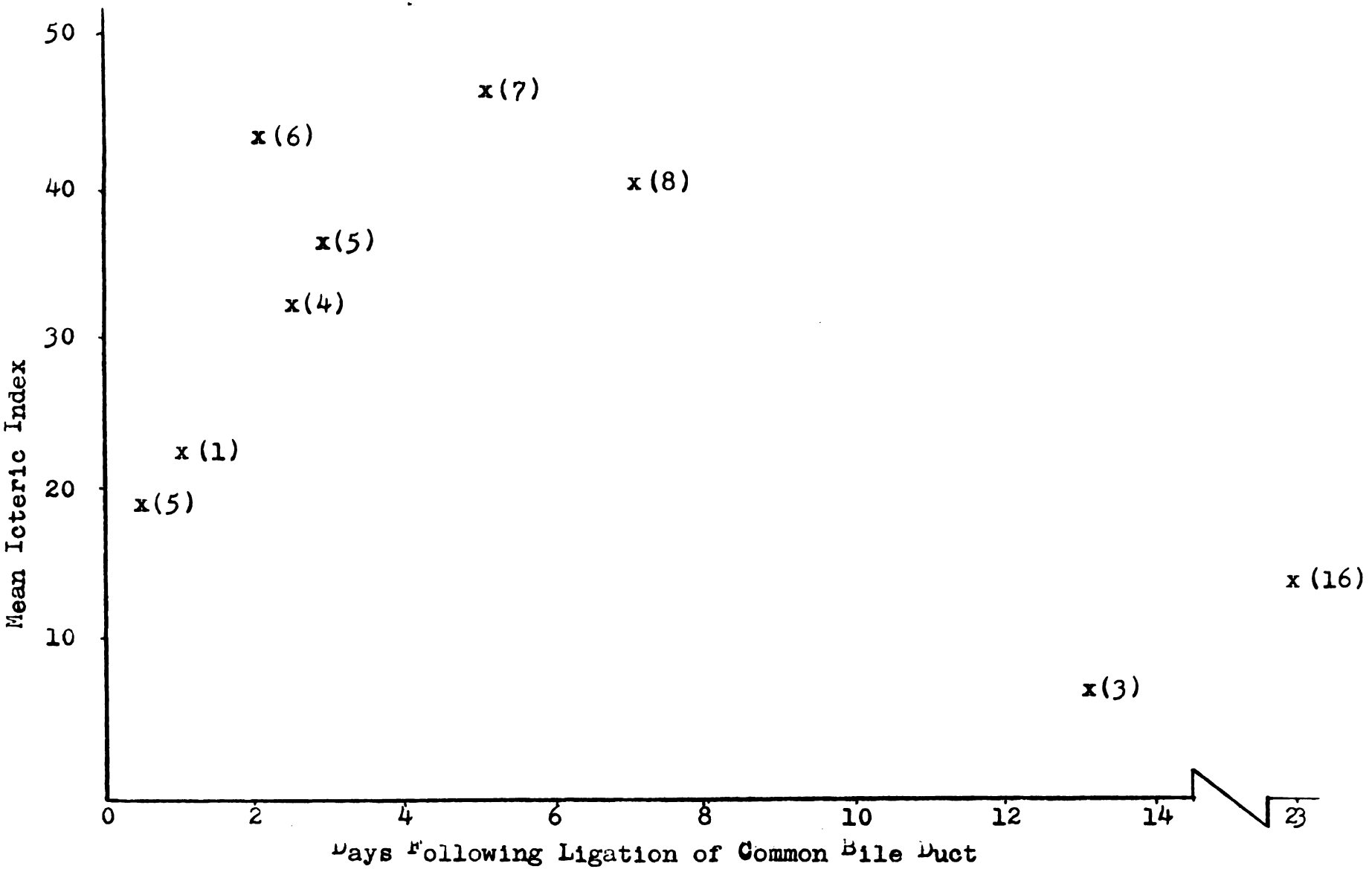

Fig.3. Graph showing the mean icteric index of rat plasma at different time intervals following ligation of the common bile duct. Numbers in parenthesis indicate number of rats represented by the adjacent point.

It will be seen in data presented in another part of this thesis that jaundice produced by the technique previously described resulted in reduced food intake, loss in body weight, thymic involution and increased liver weight. Some of the gross changes which resulted from persistent jaundice are shown in Fig. 4. This rat had been jaundiced for twenty days. This photograph shows a greatly enlarged bile duct from which approximately 20 ml. of bile were aspirated at the time of autopsy. The liver was enlarged and all of the lobes with the exception of the right lobe were discolored with bilirubin. This discoloration seen in most tissues was most pronounced in the subcutaneous connective tissue. The kidneys were colored green, presumably because of biliverdin deposition. Although it cannot be seen in this photograph, the thymus had undergone extreme involution.

Figs. 5 and 6 show photomicrographs of bile ducts taken from a normal and a jaundiced rat respectively. The thickness of the wall of the duct is much greater in the icteric rat, indicating that the duct had grown as a result of pressure and that the increase in lumen diameter did not represent simple dilatation. The increased thickness of the bile duct wall was due primarily to a great increase in growth of white fibrous connective tissue in the wall of the duct.

The histological changes in the liver which resulted from ligating the common bile duct can be seen by comparing the photomicrographs of normal (Fig. 7) with those of jaundiced

Fig.4. Photograph showing enlarged liver and bile duct in a rat which had been jaundiced for twenty days.

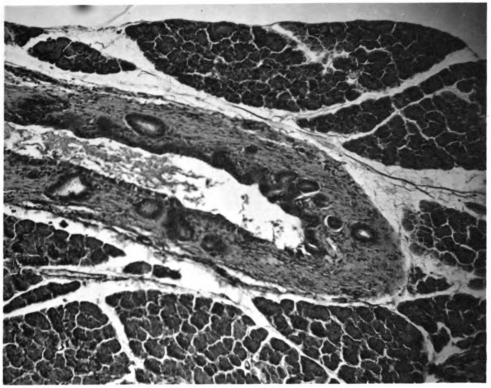

Fig.5. Common bile duct X 120. stained with hematoxylin and eosin. Section taken from normal rat in region of the pancreas.

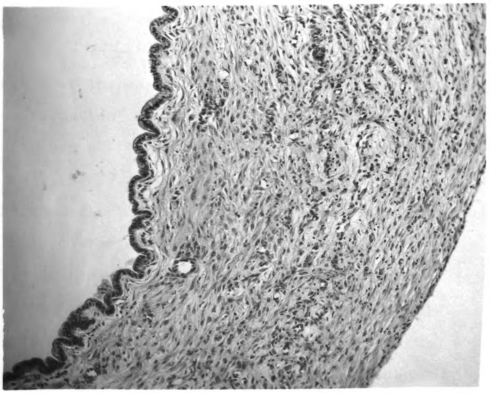

Fig.6. Common bile duct X 120, stained with hematoxylin and eosin. Section taken from a rat which had been jaundiced for ten days.

liver (Figs. 8, 9, 10). Fig. 7 shows the normal parenchymal tissue. In the center of this section is seen a normal bile duct, hepatic artery and interlobular vein. Fig. 8 is a photomicrograph of liver from a rat which had been jaundiced for twenty days. The outstanding characteristic of this tissue is the greatly increased number of bile ducts. Many of these bile ducts are larger than those seen in normal liver. Fig. 9 shows the biliary cirrhosis in the liver of a rat which had been jaundiced for six days. This cirrhosis is characterized by increased fibroblastic proliferation in the region of the bile ducts and a laying down of connective tissue in this region.

Fig. 10 shows an organized thrombus located in a central vein of a jaundiced liver. Streaks of fibrin deposits, including polymorphonuclear leucocytes, can be seen together with the attachment of the thrombus to the wall of the vein. The formation of this thrombus was probably due to venous occlusion as a result of increased pressure on the biliary system. Thrombus formation, biliary cirrhosis and an increased number of bile ducts are all typical of obstructive jaundice (Boyd, 1945 and Horrall, 1938).

There was little if any actual difference in the morphology or staining characteristics of the adrenals between normal (Fig. 11) and jaundiced (Fig. 12) rats. The differences seen in these photomicrographs are believed to reflect differences in regions where the sections were taken rather than actual changes in the gland itself. Histological examination of the

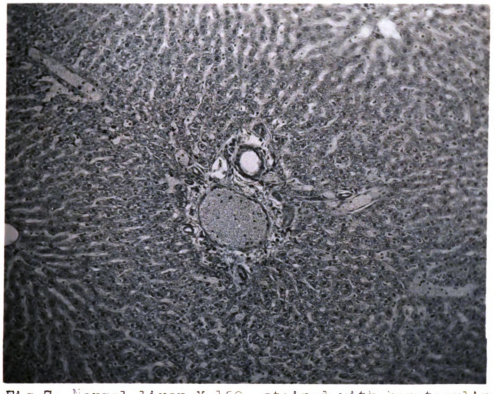

Fig.7. Normal liver X 120, stained with hematoxylin and eosin.

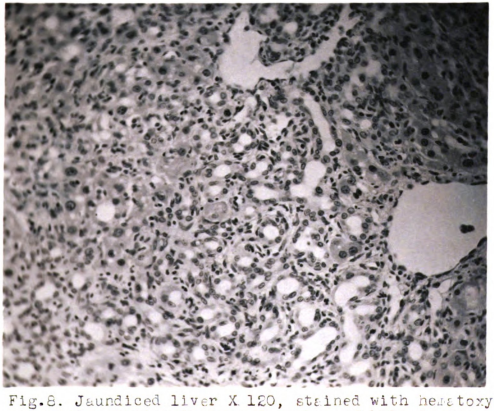

Fig.8. Jaundiced liver X 120, stained with hematoxylin and eosin showing increased number of bile ducts.

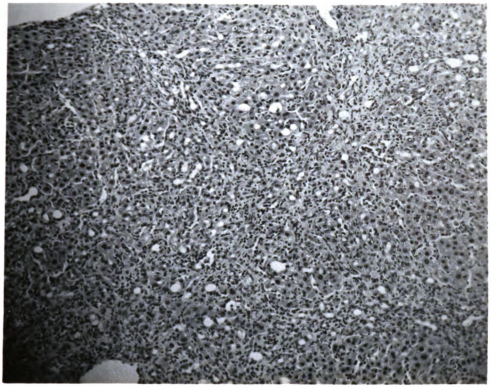

Fig.9. Jaundiced liver X 120, stained with hematoxylin and eosin showing biliary cirrhosis.

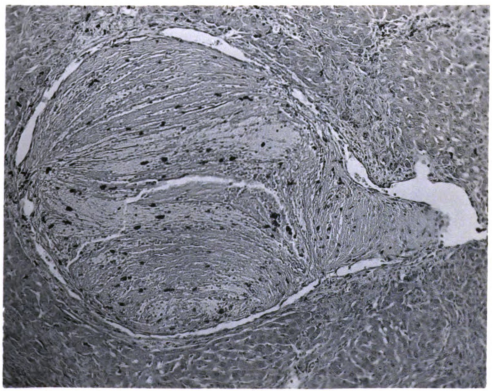

Fig.10. Jaundiced liver X 120, stained with hematoxylin and eosin showing organized thrombus and attachment to vein.

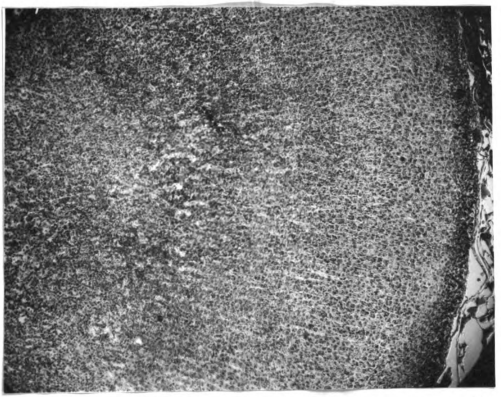

Fig.11. Normal adrenal X 90, stained with sudan IV and hematoxylin.

Fig.12. Jaundiced adrenal X 90, stained with sudan IV and hematoxylin.

adrenals from twenty rats, ten of which had been jaundiced for six days, showed no apparent changes in the thickness or lipid content of any of the three zones of the cortex.

Measure of Adrenal Cortical Function by Formation of Granuloma Tissue Around Cotton Pellets. The methods of Meier et al. (1950) was used in experiments II and III to study adrenal cortical activity in rats. This method is based on the ability of the 11-oxycorticosteroids to prevent connective tissue deposition in response to mechanical irritation. In this procedure, cotton pellets of known weight, between 4.5 and 6.0 mg. each, were implanted in the subcutaneous tissue of the back of each rat. Several days later the encapsulated pellets were removed and weighed. The initial dry weight of the cotton was subtracted from the wet weight of the encapsulated pellet, leaving only the weight of the formed granuloma. This procedure has been used successfully by Meyer et al. (1953) to study adrenal cortical activity in pregnant and lactating rats. Similar methods using various irritants have been employed by other workers. For specific details of the histology of connective tissue growth the interested reader is referred to the discussion of Ingle and Baker (1953).

Experiment I. Effects of Experimental Jaundice on Thymus and Adrenal Weights.

Procedure:

Twenty-four male rats of the Carworth strain were used in this experiment to determine the effects of experimental jaundice on adrenal cortical activity. These rats were divided into three groups of eight rats each. The animals of the first group (group 1) were sham-operated and allowed to eat ad libitum. The common bile duct of each of the rats in group 2 was ligated and these rats were also permitted to eat ad libitum. The rats of group 3 were sham-operated and pair-fed to group 2. All of the rats were sacrificed on the eighth postoperative day by overdosage with ether. A blood sample was withdrawn from each of the animals of group 2 and the icteric index was determined. The thymus and adrenal glands were removed and weighed on a Roller-Smith balance.

RESULTS:

The results of this experiment are summarized in Tables II and III. It can be seen from the data in Table II that the jaundiced rats (group 2) lost considerable weight and consumed less food than the sham-operated controls (group 1). The sham-operated rats (group 3) which were pair-fed to the icteric rats (group 2) exhibited a similar weight loss.

The data in Table III show thymus weight was reduced in the jaundiced rats (group 2) about 50 percent and not at all

in the sham-operated controls (group 1) or pair-fed controls (group 3). It can also be seen that there were no significant differences between the adrenal weights of the three groups of rats.

CONCLUSIONS:

It is apparent from these data that experimental jaundice (group 2) is accompanied by reduced food intake and reduction in body weight. Since the sham-operated rats of group 3 which were pair-fed to the jaundiced rats (group 2) showed similar weight reductions, this loss of body weight of the jaundiced rats (group 2) must be attributed in large part if not entirely to the reduced food intake. It can be seen in Table III that jaundice reduced thymus weight by about 50 percent. This cannot be attributed to reduced food consumption since the rats which were pair-fed (group 3) to the jaundiced group exhibited thymus weights similar to those of the sham-operated rats (group 3) allowed to eat ad libitum.

Table II. Effects of Experimental Jaundice on Body Weight and Food Consumption

Group and no of rats	Treatment	Av. Body Weight Initial gm.	Av. Body Weight Final gm.	Av. Food Intake gm. /rat/ day	Av. Icteric Index
1(8)	Sham Operated Fed ad libitum	270.6±5.9*	272.1±7.4*	9.07	
2(8)	Bile Duct Ligated Fed ad libitum	273.8±4.3	237.5±4.3	6.75	33.1±6.5*
3(8)	Sham Operated Pair-fed to group 2	274.4±6.3	246.8±5.6	6.75	

*Standard error of the mean = $\sqrt{\frac{d^2}{n(n-1)}}$

Table III. Effects of Experimental Jaundice on Thymus and Adrenal Weights

Group and Treatment no. of rats	Av. Thymus Weight Actual mg.	Av. Thymus Weight per 100 gm. body wt. mg.	Av. Adrenal Weight Actual mg.	Av. Adrenal Weight per 100 gm. body wt. mg.
1(8) Sham Operated Fed *ad libitum*	220.3±23.9*	80.3±7.9*	28.3±2.2*	10.5±1.2*
2(8) Bile Duct Ligated Fed *ad libitum*	108.8±14.3	41.8±6.6	31.7±3.4	13.4±1.5
3(8) Sham Operated Pair-fed to group 2	190.6±23.2	76.6±8.2	29.9±1.1	12.2±0.8

*Standard error of the mean

Experiment II. Effects of Experimental Jaundice on Thymus Weight, Adrenal Ascorbic Acid and Granuloma Formation.

Procedure:

This experiment was performed in an attempt to repeat the results of the previous experiment and to determine by other means than thymus weight whether or not there is increased adrenal cortical activity in jaundiced rats. Three groups of rats were treated similar to those of the previous experiment. Each group consisted of 10 mature female Carworth rats. Group 1 was sham-operated and allowed to eat ad libitum. The common bile duct of each of the rats of group 2 was ligated and these rats were also permitted to eat ad libitum. The animals in group 3 were sham-operated and pair-fed to group 2. Three 4.5-6.0 mg. cotton pellets were weighed individually and were inplanted subcutaneously at the time of surgery. One pellet was inserted in the middle cervical region, and one each in the left and right lateral lumbar areas. All rats were sacrificed on the sixth post-operative day by over dosage with ether. A blood sample was withdrawn from the animals of group 2 just prior to sacrifice for determining the icteric index. The liver, thymus and adrenals and the three encapsulated cotton pellets were removed from each rat and weighed on a Roller-Smith balance. One of the adrenals was immediately frozen and the adrenal ascorbic acid was determined by the method of Roe and Kuether (1943).

RESULTS:

The results of this experiment are shown in Tables IV and V. It can be seen from the data in Table IV that the jaundiced rats again consumed less food than did the controls. Also the jaundiced rats (group 2) showed body weight reductions similar to that of the pair-fed controls (group 3). These results are in agreement with those of the previous experiment. Table IV shows that there were no significant differences in the adrenal ascorbic acid levels of the three groups of rats.

In Table V it can be seen that the mean liver weights of the jaundiced rats (group 2) were significantly larger than those of the remaining two groups (1 and 3). As in the previous experiment the thymus weights of the icteric rats (group 2) were significantly smaller, weighing only about half as much as either of the other two groups (group 1 and 3). The adrenal weights of the three groups were statistically the same. The average granuloma weight was significantly smaller in the jaundiced rats (group 2) than in the two remaining groups. The mean granuloma weights from groups 1 and 3 were statistically the same.

CONCLUSIONS:

The data from this experiment substantiate those of the previous experiment insofar as the effects of experimental jaundice on food consumption, body weight, and thymus and adrenal weight are concerned. It is of interest to note that the reduction of food intake (group 3) was not sufficient

to reduce either thymus or liver weights when compared to the sham-operated group which was allowed to eat ad libitum. Thus both the thymic involution and hepatic enlargement of icteric animals can be attributed to a primary effect of the jaundice and does not represent a response to reduced food intake. The reduction in granuloma formation in the jaundiced rats (group 2) as compared to groups 1 and 3 is interpreted as a further indication of increased adrenal cortical activity in the icteric animals. Although this reduction is relatively small, it is sigificant and compares favorably with the degree of change observed in granuloma weight during pregnancy in rats by Meyer et al. (1953). It was also demonstrated that ligation of the bile duct increased the size of the liver.

Table IV. Effects of Jaundice on Body Weight, Food Consumption and Adrenal Ascorbic Acid

Group and no. of rats	Treatment	Av. Body Weight Initial gm.	Av. Body Weight Final gm.	Av. Food Intake gm. /rat/ day	Av. Icteric Index	Av. Adrenal Ascorbic Acid mg. / gland
1(10)	Sham Operated Fed ad libitum	236.9±6.2*	228.7±6.2*	10.22		82.2±2.9*
2(10)	Bile Duct Ligated Fed ad libitum	234.8±5.4	214.4±6.5	5.80	47.6±7.2*	80.4±3.0
3(10)	Sham Operated Pair-fed to group 2	230.7±5.4	215.7±7.2	5.80		80.2±1.8

*Standard error of the mean

Table V. Effects of Jaundice on Liver, Thymus and Adrenal Weight and on Granuloma Formation

Group and no. of rats	Treatment	Av. Liver Weight		Av. Thymus Weight		Av. Adrenal Weight		Av. Pellet Weight **
		Actual gm.	per 100 gm. body wt. gm.	Actual mg.	per 100 gm. body wt. mg.	Actual mg.	per 100 gm. body wt. mg.	mg.
1(10)	Sham Operated Fed ad libitum	6.97±.21*	3.05±.08*	249.1±25.0*	97.5±14.7*	51.9±1.8*	22.8±0.9*	98.2±5.2
2(10)	Bile Duct Ligated Fed ad libitum	9.06±.40	4.22±.11	120.0±13.6	55.0±4.4	48.2±2.2	22.5±1.0	85.3±3.9
3(10)	Sham Operated Pair-fed to group 2	6.59±.24	3.07±.11	198.7±16.5	92.6±8.0	49.0±1.1	22.9±0.7	102.4±4.5

*Standard error of the mean
**Wet weight of pellet minus weight of cotton.

Experiment III. Effects of Adrenalectomy on Thymic and Granuloma Response to Experimental Jaundice.

Procedure:

Thirty male Carworth rats were used in this experiment to determine whether or not a functional adrenal cortex is required for the effects of jaundice observed in the previous experiments. These rats were divided into three groups of ten each and were treated as follows: The first group was sham-operated; group 2 was bilaterally adrenalectomized; group 3 was adrenalectomized and the common bile ducts were ligated. Groups 1 and 2 were pair-fed to group 3.

Three cotton pellets were implanted subcutaneously in each of the thirty rats at the time of surgery, according to the previously described technique. All animals were sacrificed on the fifth post-operative day by overdosage with ether. The adrenals of group 1 and the thymus, liver and encapsulated pellets of all three groups were removed and weighed on a Roller-Smith balance. A sample of blood was withdrawn from the jaundiced rats (group 3) just prior to sacrifice for determining the icteric index.

RESULTS:

The results are shown in Tables VI and VII. It is apparent (Table VI) that the rats of all three experimental groups lost weight as a result of reduced food consumption. The jaundiced, adrenalectomized rats (group 3) showed a body weight loss similar to that of the intact, non-jaundiced

animals (group 1). These two groups received the same amount of food.

The thymus weighed much less in the intact animals (group 1) than in either of the adrenalectomized groups (groups 2 and 3). The thymus glands of the adrenalectomized jaundiced rats (group 3) weighed only slightly less than those of the adrenalectomized non-jaundiced animals (group 2). This difference is not statistically significant. The liver weights were larger in the jaundiced rats (group 3) than in the two remaining groups (groups 1 and 2).

The granuloma weights were statistically the same in the adrenaleotomized jaundiced rats (group 3) and in the sham-operated animals (group 1). The granuloma weights of the adrenalectomized non-jaundiced rats (group 2) were larger than those of the intact animals (group 1). It is apparent from these data that the mean survival time of the adrenalectomized rats was less in the jaundiced (group 3) than in the non-jaundiced rats. Only six of the original ten rats of the former group were alive by the fifth post-operative day.

CONCLUSIONS:

The reduced survival time in the jaundiced adrenalectomized rats (group 3) makes it somewhat to difficult to accurately interpret these data. Since thymus weights were not reduced in the adrenalectomized jaundiced rats (group 3) when compared to the intact controls (group 1), it appears likely that the adrenal cortex is required for the thymic involution which

accompanies experimental jaundice. The fact that the average granuloma weight was the same in the adrenalectomized jaundiced rats (group 3) as in the sham-operated animals (group 1) also suggests that a functional adrenal cortex is necessary for the reduced granuloma formation seen in icteric rats. Since the adrenalectomized jaundiced rats survived a shorter time than adrenalectomized non-jaundiced animals it is possible that experimental jaundice constitutes a "stress" which may increase requirements for adrenal cortical hormones.

Table VI. Effects of Experimental Jaundice on Body and Adrenal Weights in Adrenalectomized Rats

Group and no. of rats	Treatment	No. of rats alive at end of 5 days	Av. Body Weight Initial gm.	Av. Body Weight Final gm.	Av. Icteric Index	Av. Adrenal Weight Actual mg.	Av. Adrenal Weight per 100gm. body wt. mg.
1(10)	Sham-Operated Pair-fed to group 3	10	210.3±6.6*	167.5±5.8*		24.9±1.0*	15.0±0.6*
2(10)	Adrenalectomized Pair-fed to group 3	10	206.6±6.2	177.1±5.9			
3(10)	Adrenalectomized Bile Duct Ligated	6	202.0±8.5	164.0±4.1	80.6		

*Standard error of the mean

Table VII. Effects of Jaundice on Thymus and Liver Weights and on Granuloma Formation in Adrenalectomized Rats.

Group and no. of rats	Treatment	Av. Thymus Weight		Av. Liver Weight		Av. Pellet Weight ***
		Actual mg.	per 100 mg. body wt. mg.	Actual gm.	per 100 mg. body wt. gm.	mg.
1(10)**	Sham Operated	101.9±10.4*	60.1±4.3*	4.26±0.15*	2.54±0.15*	59.13±2.05*
2(10)**	Adrenalectomized	234.2±26.6	130.6±12.4	4.73±0.20	2.67±0.09	69.24±2.94
3(6)	Adrenalectomized Bile Duct Ligated	192.5±11.5	117.4±6.3	6.73±0.35	4.12±0.23	61.65±3.15

*Standard error of the mean
**Groups 1 and 2 were pair-fed to group 3 which consumed an average of 0.40 gm. of food per rat daily.
***Wet weight of granuloma minus weight of cotton

Experiment IV. Effects of Experimental Jaundice on the Survival of Adrenalectomized Rats.

Procedure:

This experiment was performed to determine whether or not jaundice affects the survival of adrenalectomized rats after a preliminary injection of cortisone. It was hypothesized that if jaundice interferes with the inactivation or excretion of adrenal steroids, adrenalectomized rats might survive longer if made jaundiced and injected with a limited amount of cortisone.

Forty mature rats were divided into four groups of ten rats each and were bilaterally adrenalectomized. The first two groups of rats were males and the other two groups were females. The common bile duct of each rat in group 2 was ligated at the time of adrenalectomy. After eight rats of each of the first two groups had died, the remaining two animals were sacrificed as a part of another experiment. Group 4 was made icteric by ligating the common bile duct at the time of adrenalectomy and was allowed to eat ad libitum, while the rats in group 3 were pair-fed to group 4. Each of the rats in groups 3 and 4 was given 10 mg. of hydrocortisone acetate subcutaneously twelve hours before surgery and 5 mg. of cortisone acetate subcutaneously immediately following surgery. Both groups were fed the Hoppert ration (Meites, 1951) and given tap water to drink. The time of survival of each rat was noted.

RESULTS:

The data are shown in Table VIII. Since the animals of

the first two groups were of a different sex and were treated differently than those of the latter two groups, valid comparisons of groups 1 and 2 with groups 3 and 4 cannot be made. It can be seen however by comparing group 1 with group 2 that the adrenalectomized rats (group 2) consumed less food and survived a much shorter period of time than did the adrenalectomized controls (group 1). It is apparent from the data in the second portion of Table VIII that when adrenalectomized rats were given cortisone and compound "F" the jaundiced adrenalectomized rats (group 4) again survived a shorter time than did non-jaundiced adrenalectomized rats (group 3) which were treated similarly.

CONCLUSIONS:

These data suggest that there are increased adrenal cortical requirements in jaundiced rats. Since the jaundiced adrenalectomized rats treated with cortisone and compound "F" (group 4) survived a shorter time than similarly treated non-jaundiced animals (group 3), it appears that the exogenous adrenal steroids were more effective in maintaining the non-jaundiced rats. Thus if the adrenal steroids are inactivated or excreted at a reduced rate in jaundiced rats, these effects may have been counteracted by increased adrenal cortical requirements. Insofar as survival time is concerned, these data do not show that adrenal cortical hormones are more effective in jaundiced than in normal rats.

Table VIII. Effects of Experimental Jaundice on the Survival of Adrenalectomized Rats

Group and no. of rats	Treatment	Av. Initial Body Weight gm.	Av. Food Consumption gm. /rat/ day	Av. Survival Time rat days
	Male Rats------Received no Cortisone or Compound "F".			
1(8)	Adrenalectomized Fed ad libitum	267.1	6.95	15.9
2(8)	Adrenalectomized Bile Duct Ligated Fed ad libitum	269.0	3.73	8.2
	Female Rats------Received Cortisone and Compound "F"			
3(10)*	Adrenalectomized Pair-fed to group 4	219.7	7.90	23.2
4(10)*	Adrenalectimized Bile Duct Ligated Fed ad libitum	214.6	7.90	10.2

*Five rats of group 3 and 1 rat in group 4 were still surviving at the termination of this experiment.

Experiment V. The Effect of Experimental Jaundice on the in vitro Inactivation of Compound "F" by Surviving Liver Slices.

Procedure:

It appeared reasonable that if the liver was damaged by experimental jaundice, this might result in reduced inactivation of adrenal cortical hormones and thus could account for the increased adrenal cortical activity previously observed. The object of this experiment, therefore, was to determine whether or not experimental jaundice reduced the rate of hydrocortisone (compound "F") inactivation by surviving liver slices. Sixteen mature male rats were used in this study. Eight of these animals (group 1) were made icteric by ligating the common bile duct. The remaining eight rats (group 2) were sham-operated and pair-fed to group 1. Both groups were fed the Hoppert ration and given tap water to drink.

The rats were sacrificed on the sixth post-operative day by decapitation. The livers were quickly removed and thin slices were prepared with a razor blade. These slices were collected in a weighing bottle containing 10 ml. of oxygenated Ringers' phosphosaline solution with compound "F" added. When approximately 2 grams of slices were collected, the contents were transferred to a 125 ml. Erylenmeyer flask. Enough phosphosaline with compound "F" was then added to the flask to make a total of 1.25 ml. of phosphosaline solution per 100 mg. of liver slices. Each ml. of Ringers' phosphosaline solution contained 3.2 mg. of compound

"F". The flask was then oxygenated, stoppered and shaken contineously in a constant temperature water bath at 38° C for three hours. At the end of three hours incubation the extraction of compound "F" was carried out according to the procedure outlined in Fig. 13. This procedure was described by Nielson (1954) and is essentially that used for the commerical extraction of steroids from adrenal glands. The method employed in preparing the Ringers' phosphosaline containing compound "F" and incubating this material with liver slices is essentially the same as that used by Schneider and Horstmann (1952).

Following the extraction of compound "F" as outlined in Fig. 13, one half of the extract of each of the sixteen livers was sent to the Upjohn Company for chromatographic analysis. The remaining portions of these extracts were polled to give a composite sample from the icteric livers and another from the non-icteric livers. These two samples were then adjusted so that each represented the same initial quanity of compound "F". The ethylenedichloride was removed from these latter two samples by distillation <u>in vacuo</u>. The two resulting residues containing the compound "F" were then dissolved in absolute alcohol and each was added to 10 ml. of cottonseed oil. The alcohol was evaporated, leaving two solutions of compound "F" in cottonseed oil. This material was then injected subcutaneously into mature rats for biological estimation of compound "F" by the following procedure:

Fifty immature male rats were divided into five groups of approximately equal mean body weights. These rats were fed the Hoppert ration and given tap water to drink. The rats of group 1 were given daily injections of 0.2 ml. of cottonseed oil. The sample resulting from the pooling of the icteric liver extracts, previously described, was divided equally among the ten rats of group 2 and given in five daily injections. The sample resulting from the non-icteric liver extracts was divided equally amoung the ten rats of group 3 and given in five daily injections. The ten rats in group 4 received an amount of compound "F" (33.01 mg.) that would be contained in the unknown samples if none of the hydrocortisone been inactivated. Group 5 was given a total dose of 6.78 mg. of compound "F". This represents the average amount of compound "F" contained in the pooled samples from the icteric (7.85 mg.) and the non-icteric (5.73) livers, as determined chemically.

RESULTS:

The results of the chemical determinations of compound "F" following the incubation of this steroid with liver slices are shown in Table IX. The data pertaining to the biological assay of this same material are shown in Table X.

It is seen in Table IX that there was a slight weight loss in both the icteric (group 1) and non-icteric (group 2) animals. These data show further that there was no significant difference between the amount of compound "F" inactivated by the liver of jaundiced (group 1) and non-jaundiced (group 2) rats.

Table X shows the data from the biological assay of the remaining compound "F" following incubation with icteric and non-icteric liver. Unfortunately only the high dose of compound "F" (group 4) proved sufficient to depress thymus weight in these animals. The thymus weights of the remaining four groups (groups 1, 2, 3, and 5) were statistically the same.

CONCLUSIONS:

It is concluded from the results of the chemical analysis that there was very little if any reduction in the rate of inactivation of compound "F" by surviving liver slices as a result of experimental jaundice. These data are believed to be highly significant since an individual chemical analysis was made for each rat. The biological data are more difficult to interpret since only the high dose of compound "F" (group 4) produced significant thymic involution. Since this dose of compound "F" is equal to that added initially to the liver slices for incubation, it is possible that inactivation occured to the same extent in both the jaundiced (group 2) and non-jaundiced (group 3) livers, as neither of these groups showed signigicant thymic involution. It is impossible to determine conclusively from these biological data whether or not more compound "F" was inactivated by the jaundiced than by the non-jaundiced liver slices. However, these biological data suggest agreement with the more conclusive chemical analysis.

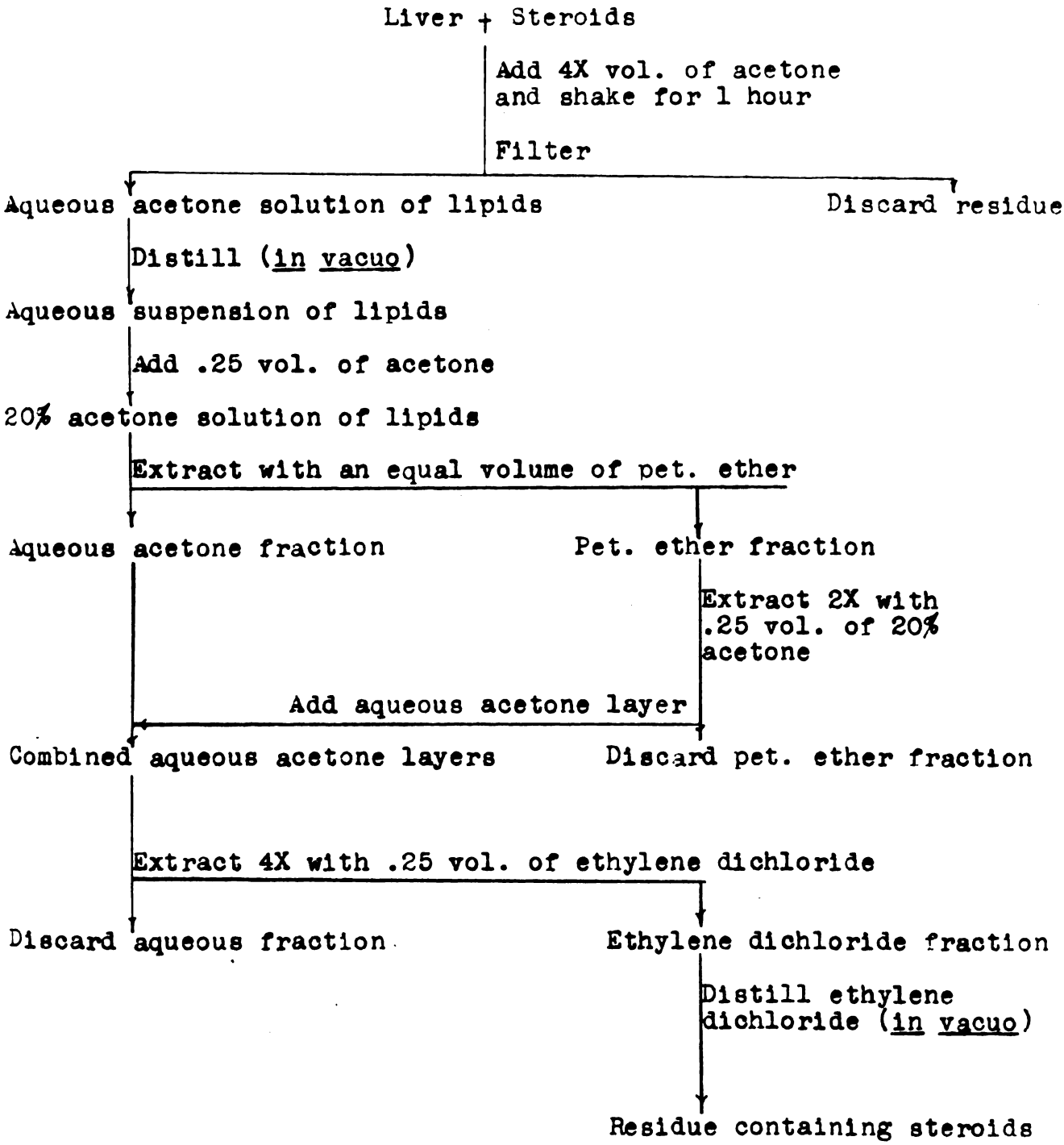

Fig. 13. An outline of the procedure used for recovering compound "F" from liver slices (Nielsen, 1954)

Table IX. Effects of Experimental Jaundice on the *in vitro* Inactivation of Compound "F"

Group and no. of rats	Treatment	Av. Body Weight Initial gm.	Av. Body Weight Final gm.	Av. Food Intake gm./rat/day	Rat no.	Cpd. "F" Inactivated (mg./100mg. Liver
					1	3.41
					2	4.48
					3	4.53
					4	4.67
					5	3.64
					6	5.08
					7	2.89
	Bile Duct Ligated				8	4.05
1(8)	Fed *ad libitum*	246.6±4.6*	229.3±5.5 *	4.1		4.09± .26*
					9	4.38
					10	4.47
					11	4.11
					12	4.57
					13	4.36
					14	2.91
					15	4.69
	Sham-Operated				16	4.71
2(8)	Pair-fed to group 1.	251.5±7.7	221.3±4.9	4.1		4.40±.10

*Standard error of the mean

Table X. Effects of Incubating Compound "F" with Icteric and Non-icteric Liver Slices on Its Ability to Depress the Thymus Weights of Mature Rats

Group and no. of rats	Treatment	Av. Body Weight Initial gm.	Av. Body Weight Final gm.	Av. Thumus Weight Actual mg.	Av. Thumus Weight per 100gm. body wt. mg.
1(10)	Cottonseed oil	177.6±7.3*	192.2±7.6*	176.1±12.0*	91.1±5.9*
2(10)	33.01 mg. cpd "F" incubated with icteric liver	178.3±8.1	210.1±9.9	181.5±14.1	86.6±5.8
3(10)	33.01 mg. cpd "F" incubated with non-icteric liver	177.8±6.6	197.9±7.8	161.9±10.4	82.6±5.7
4(10)	33.01 mg. cpd "F"	177.9±7.6	213.0±14.2	103.5±7.8	50.7±4.8
5(10)	5.73 mg. cpd "F"	178.1±4.1	203.2±4.2	162.9±10.8	80.1±5.1

*Standard error of the mean

DISCUSSION

The major conclusion drawn from this study is that increased adrenal cortical activity does accompany experimental jaundice in rats. This increased activity was demonstrated by the pronounced thymic involution and reduced granuloma formation in rats made icteric by ligation of the common bile duct.

The exact mechanism responsible for the increased adrenal cortical activity in the jaundiced rats is not clear from the studies carried out here. However, it does not appear to be the result of either increased function of the adrenal cortex or reduced corticoid inactivation. Histological examination of adrenal glands failed to reveal any notable changes in morphology or fat content as a result of jaundice. There was no significant increase in adrenal weights or reduction in adrenal ascorbic acid content. Thus it can only be concluded that experimental jaundice did not stimulate adrenal cortical function.

In the experiment involving hydrocortisone inactivation by surviving liver slices from jaundiced and non-jaundiced rats, it was found that the ability of liver slices to inactivate this adrenal hormone was not significantly reduced by experimental jaundice. The possibility still remains however that the pressure of the biliary system in jaundiced rats is sufficient to prevent the transfer of steroid metabolites into the bile and may thus interfere with their excretion and <u>in vivo</u> metabolism. An analogous situation would be the increase

in circulating level of urea following ligation of the ureters. This change would probably not be reflected in an *in vitro* study using kidney slices.

A more direct approach to this same problem was the study of plasma levels of 17-hydroxycorticoids in various clinical disorders by Perkoff et al. (1954). The average concentration of these steroids in normal individuals was found to be 13 ± 6 γ per 100 ml. of plasma. An identical circulating level of 17-hydroxycorticoids was observed by these authors in nine patients suffering from cirrhosis, hepatitis, cholecystitis and cholelithiasis. Since Perkoff et al. (1954) found no elevation of 17-hydroxycorticoids in jaundiced patients, it seems unlikely that the hepatic inactivation of these steroid substances is impaired in such patients. Thus the findings of Perkoff et al. (1954) as well as those of the present study point to the conclusion that there is probably no significant increase in adrenal cortical function or reduction in corticoid inactivation as a result of jaundice.

The data presented here also support the clinical findings of Sprague et al. (1951), who showed in sixteen jaundiced patients that the urinary excretion of corticosteroids was well within the limits of normal individuals. Injections of the minimal therapeutic dose of cortisone required for alleviating arthritis produced a sharp rise in the level of corticosteroids excreted by these patients. Sprague et al. (1951) concluded that an increased adrenal cortical function in

jaundiced patients sufficient to alleviate arthritic symptoms should have been reflected in increased excretion of corticoids. The obvious implication of these clinical results is that an increased adrenal cortical function is not the mechanism underlying the alleviation of arthritic symptoms seen in jaundiced individuals.

Del Roselli and Matteini (1949) studied the endocrine changes resulting from total occlusion or partial obstruction of the common bile duct in mature rabbits. This treatment caused the death of the animals after different time intervals. Such animals showed increased pituitary weight with disappearance of eosinophils and an increase in the number of basophils. There was a definite increase in the adrenal weights of rabbits which died from bile duct occlusion and a marked hyperplasia was noted, particularly in the fascicular zone. A marked involution of the thymus was also observed by these authors in the jaundiced rabbits. They also carried out chronic experiments in which the rabbits survived three months to one year following bile duct obstruction. In these animals, increased adrenal weights were seen in some, but not in all the jaundiced rabbits. The fasciculata and glomerulosa zones of the adrenals in the rabbits with chronic biliary stasis showed a great increase in fat content and the reticular zone showed signs of degeneration.

In the present study there were no apparent changes in the adrenals of the jaundiced rats. Apart from rats not belonging to the same species as rabbits, the rats in the present experi-

ments were jaundiced only about seven days before sacrifice as compared to several months in the rabbit experiments of Del Roselli and Matteini (1949). Whether or not the accumulation of fat in the fasculata and glomerulosa of the rabbit adrenals seen by Del Roselli and Matteini (1949) indicates increased adrenal cortical function is difficult to determine. In acute experiments the loss of lipid rather than the deposition of such material is taken as evidence of increased adrenal cortical function (Selye, 1950).

It appears from the results of this study and those of other workers that neither increased adrenal cortical function or reduced liver inactivation contributes to the increased adrenal cortical-like activity seen in jaundiced rats or to the alleviation of arthritic symptoms seen in jaundiced patients. It might seem logical to conjecture that either (1) an increased sensivity of body tissues to adrenal steroids accompanies jaundice or (2) an extra-adrenal substance, with effects similar to those of the adrenal steroids in respect to thymus involution, granuloma formation and relief from arthritis may be elicited by experimental or spontaneous jaundice. This may arise from the bile salts or pigments. However some of the present data do not seem to support either of these conjectures. Thus it has been shown that injections of cortisone acetate of compound "F" did not extend survival to a greater extent in jaundiced adrenalectomized rats than in rats which were merely adrenalectomized. However, this was probably not a fair trial of the efficacy of

adrenal hormones during jaundice and should have been repeated in intact jaundiced rats. Rats which are both jaundiced and adrenalectomized may not be normally responsive to adrenal cortical hormones when compared to intact jaundiced animals. The second possibility seems even more remote as a result of the data showing that jaundice failed to reduce thymus weight or decrease granuloma formation in adrenalectomized rats. In short, intact adrenals appear to be essential, at least in a permissive role, for the thymic and granuloma reactions seen in the jaundiced rats. The foregoing also makes it extremely doubtful that the accumulation of bile salts or pigments can account for the observed phenomena, since they are present just as well in adrenalectomized as in intact animals. Furthermore, Hench (1938a) has failed to observe any notable alleviation of arthritic symptoms by injecting large doses of bile salts into arthritic patients. The first possibility and others which have not yet occured to the writer remain to be investigated.

SUMMARY

1. Experimental jaundice was produced in rats by ligating the common bile duct. The effects of the resulting jaundice on adrenal cortical activity, adrenal function and liver inactivation of hydrocortisone (compound "F") were studied.
2. Ligation of the common bile duct of rats resulted in a marked increase in the icteric index of the plasma. The average icteric index reached a maximum of 47 on the fifth postoperative day and then declined slowly due to the recovery of some of the animals. The icteric index was found to average only 3.9 in normal control rats. Gross and microscopic changes typical of obstructive jaundice were seen in the rats made jaundiced by this procedure.
3. Twenty-four male rats were used in an experiment to determine the effects of experimental jaundice on adrenal cortical activity. On the eighth day following bile duct ligation the animals were autopsied and it was found that the thymus of the jaundiced rats had undergone marked involution. This effect could not be attributed to the surgery or reduced food consumption since a sham-operated, pair-fed group of rats failed to show thymic involution. It was noted however, that jaundice was accompanied by decreases in food consumption and body weight. There was no significant difference in the weight of the adrenals between the jaundiced and non-jaundiced rats.

4. In another experiment, thirty rats were made jaundiced and were sacrificed on the sixth post-operative day. Three 4.5-6.0 mg. cotton pellets were implanted subcutaneously in each of the rats at the time of bile duct ligation or sham-operation. These pellets plus the granuloma formed around the cotton were removed and weighed at the time of sacrifice. In addition, the thymus, liver, and adrenals were weighed and the adrenal ascorbic acid was determined. Reduced granuloma formation and a decrease in thymus weight were found in the jaundiced rats but not in the sham-operated, pair-fed controls. There was increased liver weight in the icteric rats but no significant differance occured between the adrenal ascorbic acid levels of the jaundiced and non-jaundiced animals.
5. Thirty male rats were used in another experiment to determine whether or not a functional adrenal cortex was required for the previously observed effects of jaundice on thymus weight and granuloma formation. Some of the rats which were both adrenalectomized and jaundiced succumbed, making the data somewhat difficult to interpret accurately. It was observed however, that thymus involution and granuloma inhibition did not occur in the jaundiced rats which were adrenalectomized. This suggests that a functional adrenal cortex is required for the increase in adrenal cortical activity seen in experimental jaundice in rats.
6. The effects of experimental jaundice on the survival of adrenalectomized rats given a single dose of compound "F" was studied

in forty mature rats. These rats were given a large dose of compound "F" just prior to adrenalectomy. In addition, the common bile ducts of some of the animals were ligated. It was observed that the jaundiced adrenalectomized rats given compound "F" did not survive as long as the similarly treated non-jaundiced animals. It was concluded that jaundice probably does not interfere with the inactivation or excretion of this adrenal steroid. An increased utilization of the adrenal steroids may be indicated.

7. An experiment was performed on sixteen rats to determine whether or not experimental jaundice affects the <u>in vitro</u> inactivation of hydrocortisone (compound "F") by surviving liver slices. Eight of these rats were made icteric by ligation of the common bile duct. The remaining eight animals were sham-operated and pair-fed to the jaundiced group. On the sixth post-operative day the rats were sacrificed, the livers removed, and liver slices were prepared with a razor blade. Slices from each of the sixteen livers were incubated for three hours in Ringers' solution containing a known quantity of compound "F". Following this incubation period, the remaining compound "F" was extracted and determined chemically (by paper chromatography) and by means of a biological assay based on thymic involution. The chemical analysis showed that icteric and non-icteric liver slices inactivated similar quantities of compound "F". The biological assay showed that a large quantity of compound "F" was inactivated by both the jaundiced and non-jaundiced liver and that

the effects of both on thymus weight were similarly negative. It was concluded that experimental jaundice does not result in decreased inactivation of this adrenal cortical hormone.

8. It is suggested that thymic involution and reduced granuloma formation in rats and perhaps the disappearance of arthritic symptoms in jaundiced human patients are brought about by increased sensivity of the body tissues to adrenal steroids or to other mechanisms not yet determined.

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