by the summary in Table I no such gains were obtained. It is concluded that if fixation occurs it is too weak to be detected by the Kjeldahl method. The possibility that the slight gains occasionally observed possess significance is now being tested using the much more sensitive isotopic method.

Summary. Fixation of molecular nitrogen could not be induced in free-living cultures of *Rhizobium* by supplying them with extracts of root nodules containing the hemoglobin-like pigment. Nine experiments were made during 2 growing seasons. Variations in technique included: species of bacteria; source of nodular extract; addition of oxalacetic, α-ketoglutaric and citric acids; and method of preparing the extract. Analyses of the results showed that gains of 0.2-0.3 mg N were required for statistical significance, but even these slight increases were not consistently obtained.

16048

Effect of Induced Liver Cirrhosis on the Reproductive System of the Male Rat.*

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While it has been shown repeatedly that the physiology of reproduction is dependent upon the maintenance of a normal pituitary-gonadal balance, it is also becoming increasingly evident that intact liver function is essential to the normal metabolism of the sex steroid hormones. The results of *in vitro* experiments performed by numerous investigators have provided ample evidence that the liver is the principal organ responsible for the inactivation and removal from the blood of the natural estrogen of the body (Silberstein *et al.*,[1] Zondek,[2] Engel and Navratel,[3] Heller *et al.*,[4] Heller,[5] and Engel,[6]). Inactivation of endogenous estrogens by the liver has been reported by numerous workers using varying techniques. Talbot[7] induced liver damage in female rats with carbon tetrachloride in alcohol and found an increase in uterine weight of 200% by the third day of the experiment. From this he concluded that the poisoned animals were exposed to an increased concentration of blood estrogen because of the impaired inactivating capacity of the liver.

Other experiments (Golden and Severinghaus,[8] Biskind and Mark,[9] Biskind,[10] Krichesky, Benjamin and Slater,[11]) were devised to divert the gonadal hormones directly into the portal or into the systemic circulation. When the portal route was used the hormones were promptly inactivated, as shown by failure to maintain the sex accessories of castrate animals, whereas the hormones escaped inactivation when they drained directly into

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1 Silberstein, F., Engel, P., and Molnar, K., *Klin. Wchnshr.*, 1933, **12**, 1693.

2 Zondek, B., *Skand. Arch. fur Physiol.*, 1934, **70**, 133.

3 Engel, P., and Navratel, E., *Biochem. Z.*, 1937, **292**, 434.

4 Heller, C. G., Heller, E. J., and Severinghaus, E. L., *Am. J. Physiol.*, 1939, **126**, 530.

5 Heller, C. G., *Endocrin.*, 1940, **26**, 619.

6 Engel, P., *Endocrin.*, 1941, **29**, 290.

7 Talbot, N. B., *Endocrin.*, 1941, **25**, 290.

8 Golden, J. B., and Severinghaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

9 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

10 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 266.

11 Krichesky, B., Benjamin, J. A., and Slater, C., *Endocrin.*, 1943, **32**, 345.

the systemic circulation and consequently the sex accessories were maintained. The usual method was to transplant the gonad into the mesentery to facilitate drainage into the portal vein or to transplant the gonad into the axilla or body wall to prevent portal drainage. Steroid hormone pellet implants in the spleen of castrate rats was the method favored by Biskind and his associates.

Further indication of hepatic inactivation is provided by the study of steroid hormone metabolism in the presence of experimental or clinical liver damage. Extensive liver disease seems to impair hormone inactivation so that estrogenic effects are usually intensified Pincus and Martin[12] report an 80% increase in estrogen effectiveness in rats with experimental liver damage. Human observations (Edmondson *et al.*,[13] Glass, *et al.*,[14,15] Gilder and Hoagland,[16] and others) imply that acute or chronic liver disease, if extensive enough, may be associated with impairment of steroid hormone metabolism. Such disease may be followed by impairment of testicular function or the development of the full blown syndrome of testicular atrophy, gynecomastia and torso alopecia. The latter syndrome has been ascribed by one of us (Glass) to the effects of circulating biologically active estrogens emanating from failure of hepatic inactivation. To test this thesis it was deemed advisable to study the sex organs of male rats with experimental liver damage. The spontaneous effects of liver damage on the sex organs of the experimental animal have not been adequately investigated.

Method. Eighty-four mature male Wistar albino rats weighing from 150 to 210 g were used and were maintained on Rockland rat pellets and lettuce. All animals were intubated thrice weekly and given various amounts of carbon tetrachloride in 50% alcohol. Two experiments were carried out.

Experiment I. A pilot experiment using 35 animals was undertaken to determine the amount of carbon tetrachloride that would produce maximum hepatic damage with minimum mortality. The animals were divided into 3 approximately equal groups: Group A received 0.025 cc carbon tetrachloride in alcohol in doses of 0.5 cc; Group B received 0.05 cc in doses of 0.5 cc; Group C received 0.10 cc in doses of 1.0 cc. Animals from each group were sacrificed at selected intervals. Biopsies were carried out during the course of the treatment which continued for as long as 12 weeks in some animals. Macroscopic examination of the liver was made at autopsy and portions of the liver and kidney were prepared for histologic study.

Experiment II. Having determined an adequate dosage of carbon tetrachloride, a second experiment was carried out to determine the effects of hepatic injury on the male reproductive system. Forty-nine animals were used, 17 either died or were sacrificed for histologic material during the course of the experiment and the remaining 32 provided the quantitative data. The experimental animals were intubated 3 times per week with 0.05 cc carbon tetrachloride in 50% alcohol solution in doses of 0.5 cc. Five groups were employed: Group 1, animals serving as controls without treatment; Group 2, animals intubated over a period of 22 days; Group 3, animals intubated over a period of 43 days; Group 4, animals intubated over 60 to 80 days; Group 5, animals intubated for 88 days and then maintained without treatment for 40 days.

All animals were weighed before and after the experiment. The sex accessories were carefully dissected free of surrounding connective tissues and were weighed on a torsion balance. The testes were also removed and weighed. These structures and the liver and kidneys were preserved for histologic study.

Results. In the first experiment the mortality rate was greatest in those animals receiving the largest dose of carbon tetrachloride. Within a few days they appeared sickly; the

[12] Pincus, G., and Martin, D. W., *Endocrin.*, 1940, **27**, 838.

[13] Edmondson, H. A., Glass, S. J., and Soll, S. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 97.

[14] Glass, S. J., Edmondson, H. A., and Soll, S. N., *Endocrin.*, 1940, **27**, 749.

[15] Glass, S. J., Edmondson, H. A., and Soll, S. N., *J. Clin. Endocrin.*, 1944, **4**, 54.

[16] Gilder, H., and Hoagland, C. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 62.

TABLE I.
Effect of Carbon Tetrachloride Intubation of the Weight of the Sex Accessories in the Male Rat (0.05 cc CCl_4 in 50% Alcohol Administered 3 × Weekly).

Group No.	No. of animals	Duration of treatment, days	Avg body wt, g	Avg sex acces. wt, mg	Avg testis wt, mg
1	8	0	163	845	1841
2	6	22	160	637	1921
3	7	43	154	390	1663
4	7	60-80	155	278	1840
5*	4	88+40	207	476	1586

* In Group 5 carbon tetrachloride was administered for 88 days. The animals were then maintained for 40 days without treatment before they were sacrificed.

fur was rough; the backs were arched and they lost weight rapidly. Most of these were sacrificed in order to obtain fresh tissues for histologic study. The mortality among the other 2 groups was almost the same (approximately 20%) and only few of the surviving animals showed observable evidence of illness. Liver necrosis appeared in the animals receiving 0.025 cc of carbon tetrachloride by the 19th day and by the 11th day in the 0.05 cc group. Cirrhosis was present in the 0.025 cc group by the 58th day and by the 44th day in 0.05 cc group. The latter dose, therefore, produced maximum hepatic injury in the shortest time with no greater mortality than in the .025 cc group.

The liver manifested a series of progressive architectural changes not unlike that seen in human cirrhosis, that is, cloudy swelling and fatty infiltration in early stages, followed by central necrosis of the lobule with attempts at healing in the longer term experiments. Fibrosis, as well as gross nodularity was evident after 44 days of carbon tetrachloride feeding. In animals with more advanced liver failure, ascites was usually present.

From the onset of carbon tetrachloride intubation the kidneys of all animals became pale in color and later took on a yellowish cast. In the long term experiments the color was usually a chocolate brown. The histologic picture was remarkably constant with an early cloudy or albuminoid swelling followed by more severe swelling of the cells and accompanied by a coarsening of the reticulum and a clouding or granulation of the cytoplasm. This persisted throughout the experiment and was nearly constant at all dosage levels.

In the second experiment the pertinent data are given in Table I. The average weight of the sex accessories (including seminal vesicles, coagulating gland, ampullary gland, and the lateral, dorsal and ventral lobes of the prostate) was 845 mg in the untreated animals. After 22 days of treatment with 0.05 cc carbon tetrachloride the average weight of the accessories was 637 mg or approximately a 25% decrease. After 43 days of treatment the average weight was decreased by 53% to 390 mg. The group receiving treatment for 60 to 80 days showed accessories weighing only 278 mg, a 67% decrease below the untreated controls. The final group treated for 88 days and then maintained for 40 days without treatment before being sacrificed showed partial recovery in the weight of the accessories. In this group the accessories averaged 476 mg, a decrease of 44% below the controls and an increase of 70% over the group treated for 60 to 80 days and sacrificed immediately thereafter.

Although there was striking atrophy of the sex accessories following liver damage by carbon tetrachloride, yet the results indicate that the testicles were not so greatly affected. There was great variation in testicular weight among animals of all groups and because the number of animals used was small the data given in Table I are not considered significant.

Histologic examination of the testes revealed no striking architectural disorganization of the seminiferous tubules. Spermatogenesis was not greatly impaired in the majority of animals. In only 2 of 32 testes examined was marked testicular damage observed. This was indicated by absence of mature sperm and marked atrophy of spermatogenic cells.

Discussion. The data presented indicate that the oral administration of carbon tetrachloride produces hepatic damage and results in atrophy of the secondary sex organs of the male rat as indicated by reduction of weight part of which may be accounted for by the atrophy of bled tissue and part by possible reduction in the amount of the secretions of these organs. This treatment causes no significant weight changes in the testes. Healing of the liver damage promptly follows withdrawal of the poisoning agent and is accompanied by partial recovery of the weight of sex accessory organs 40 days after cessation of carbon tetrachloride administration.

In explaining these data two possible hypotheses suggest themselves; (1) that hepatic damage and sex accessory atrophy may be produced by the toxicity of the carbon tetrachloride and (2) that liver damage due to carbon tetrachloride may cause failure of hepatic inactivation of endogenous estrogens resulting in an increased level of circulating estrogens.

In the first instance, it must be admitted that direct toxic effects of carbon tetrachloride on the accessories have not been eliminated in these experiments. The atrophy of the secondary sex organs may well be due to a direct toxic action of the poison in a manner similar to the damage produced in the liver. However, it would be remarkable that such toxicity would affect only the accessory organs and not the testes, especially since the latter have been reported to be highly sensitive to noxious agents. It is surprising that only 2 animals of 32 examined show degenerative changes in the testes and no significant weight loss. The accessory organs, on the other hand, in all animals, exhibit a weight loss ranging from 25 to 67% below untreated animals. If this atrophy is due to a direct toxicity of carbon tetrachloride, then these data suggest that in the doses employed, the poison affects the sex accessory organs preferentially and has little or no effect on the testes. A difference in threshold of these organs obtains some support from the findings of Simpson and Evans[17] that the sex accessories are less resistant to androgen deprivation than the testicular tubules.

It should be pointed out also that in some unpublished experiments carried out in this laboratory in which hepatic damage was induced in rats by a low protein diet (4% casein with vitamin supplements), atrophy of the prostate and seminal vesicles was observed in the males. These results, without the use of a toxic agent, were qualitatively similar to those after carbon tetrachloride feeding. It should be recognized, however, that deficiency diets, by interference with normal metabolism, may serve as toxic agents in themselves.

A second possible explanation, that liver damage induced by carbon tetrachloride feeding may prevent hepatic inactivation of endogenous estrogens resulting in an increased level of circulating estrogens, may account adequately for the results reported here.† A high level of circulating estrogens, especially in the "free" form as suggested by Glass, Edmundson and Soll may induce secondary sex atrophy either by a direct inhibition of these organs or by inhibition of pituitary gonadotropin secretion. This view is supported by the findings of Morrione[18] that doses of estrogens insufficient to produce testicular damage in normal rats resulted in severe testicular damage when given to animals with livers damaged by carbon tetrachloride feeding. He suggested that these low doses were effective only in the latter group of animals (with damaged livers) because circulating estrogen levels in them were increased.

Healing of liver injury and recovery of the sex accessories following withdrawal of carbon tetrachloride feeding may be explained by either hypothesis. Withdrawal of the poison

[17] Simpson, M. E., Li, C. H., and Evans, H. M., *Endocrin.*, 1944, **35**, 96.

† That circulating endogenous estrogens after hepatic injury are increased is supported by unpublished data from this laboratory in which bioassay showed approximately a thousand-fold increase in urinary estrogens excreted by female guinea pigs with carbon tetrachloride-damaged livers over that excreted by normal animals.

[18] Morrione, T. G., *Arch. Path.*, 1944, **37**, 39.

permits healing and recovery by removing the deleterious agent or by healing of the liver and a return to normal steroid metabolism with consequent inactivation of endogenous estrogens.

Summary. Pathologic changes in the liver and kidneys of male rats fed carbon tetrachloride is described. Striking atrophy of the secondary sex organs but no significant changes in weight of testes follows variable periods of carbon tetrachloride feeding. Withdrawal of the poisonous agent results in healing of the liver and partial weight recovery of the sex accessories.

16049 P

Catheterization of the Coronary Sinus and the Middle Cardiac Vein in Man.*

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A technique of intravenous catheterization of the coronary sinus has been developed recently in intact dogs by Goodale and Lubin,[1] using the Cournand intravenous catheter.[2] This method has been employed by Eckenhoff and co-workers[3] to measure coronary blood flow, using the nitrous oxide method developed by Kety and Schmidt for the determination of cerebral blood flow.[4] The procedure appears to be less hazardous than the method in intact dogs previously reported by Harrison and co-workers,[5] which involved the use of a brass balloon cannula. In a combined study of 20 different dogs,[1,3] as many as 7 catheterizations and 4 duplicate measurements of coronary flow have been performed on the same dog at monthly intervals. These developments suggested that a similar procedure might be followed in the determination of coronary blood flow in man. This paper is a preliminary report of 9 successful catheterizations of the coronary vessels in man.

Methods. Most of the patients studied had congenital heart disease. Consequently, catheterization of the heart of these individuals was primarily undertaken to obtain information concerning the nature of the cardiac anomalies.[6,7,8] Only one subject, (No. 7, Table I), had a normal heart. For catheterization of the right heart the technique of Cournand[2] was followed, using standard No. 7 catheters. Passage of the catheter into the coronary sinus or the middle cardiac vein was verified by (a) fluoroscopic control, (b) recording of pressures, and (c) determinations of blood oxygen and carbon dioxide contents. It was assumed that the coronary sinus had been successfully intubated if the systolic pressures were below ventricular and above auricular systolic pres-

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† First Lt., Medical Corps, A.U.S., Army Chemical Center, Maryland.

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2 Cournand, A., *Fed. Proc.*, 1945, **4**, 207.

3 Eckenhoff, J. E., Hafkenschiel, J. H., Harmel, M., Goodale, W. T., Lubin, M., and Kety, S. S., in press.

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6 Bing, R. J., Vandam, L. D., and Gray, F. D., Jr., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 107.

7 Bing, R. J., Vandam, L. D., and Gray, F. D., Jr., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 121.

8 Bing, R. J., Vandam, L. D., and Gray, F. D., Jr., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 323.