The unidentified spots all disappeared following the acid hydrolysis of this fraction. The chromatogram then showed further increases in the concentrations of glutamic and aspartic acids, but other amino acids were also increased. There was no evidence that basic amino acids were present. Their absence is consistent with the fact that Amberlite I-R-4 is an acid-binding resin.

Conclusions. An enzymatic casein hydrolysate was found to contain an agent which produced significant changes in the electrical activity of the rabbit brain when administered under standard test conditions. The agent appeared to be a protein fragment which is adsorbed by Amberlite I-R-4 and yields large amounts of glutamic and aspartic acids when destroyed by acid hydrolysis.

Received June 26, 1950. P.S.E.B.M., 1950, v75.

Influence of Age on Hepatic Response to Choline and Cystine Deficiency in the Rat.* (18268)

PHILIP HANDLER AND RICHARD H. FOLLIS, JR.

From the Department of Biochemistry and Nutrition, Duke University School of Medicine, Durham, and the Department of Pathology, Johns Hopkins University School of Medicine.

During the past 15 years there has accumulated a considerable body of literature describing the hepatic sequelae of choline and cystine deficiencies. Unfortunately, this literature has been complicated by the fact that the many investigators in this field have employed rats of different strains and ages as well as diets of quite different composition. The present paper describes an attempt to determine the extent to which the initial age of the rat modifies the hepatic response to choline and cystine deficiency in one strain of rats fed one variety of deficient diet.

Experimental. The rats used in these studies were males of the Vanderbilt strain (1). All animals were grown to the desired weight on a commercial feed, housed in individual cages and offered the experimental rations and water *ad libitum*. The basal diet consisted of acid and alcohol washed casein 5, corn starch 54, cottonseed oil 10, cod liver oil 4, shortening 20, salts(4) 4, cholesterol 0.5, cystine 0.6 and choline chloride 0.8. Deficient diets were prepared by simply omitting one of these constituents. In addition, each diet contained the following in mg/kilo of diet mixture: Thiamine 5, riboflavin 8, pyridoxine 5, calcium pantothenate 30, *p*-aminobenzoic acid 75, α-tocopherol 25, biotin 1, pteroylglutamic acid 2, naphthohydroquinone acetate 5, niacin 50. These diets were chosen because of the considerable body of previous experience(2,3) with choline and cystine deficiency in rats fed such rations. With each deficient diet, rats of 3 ages were employed whose initial weights were 50, 150 and 300 g. Since previous experience had indicated that older rats were comparatively resistant to these deficiencies, the experiment was planned in such fashion that, for each of the 3 diets, a representative group of the older rats was sacrificed at about the time the youngest rats on the diet had died. The remaining rats were maintained on the diets and, finally, those which survived were sacrificed after they had been on the diet for 270 days. All livers were fixed in formalin, sectioned and stained with hematoxylin and eosin. On ex-

* This study was supported in part by grants from the Nutrition Foundation and the Duke University Research Council. Our thanks are due also to Merck and Co. for the crystalline vitamins used and to Miss Ludie Avery for technical assistance.

1. Wolfe, J. M., Bryan, W. R., and Wright, A. W., *Am. J. Cancer,* 1938, v34, 352.

2. Handler, P., and Follis, R. H., Jr., *J. Nutrition,* 1948, v35, 669.

3. Handler, P., and Dubin, I. N., *J. Nutrition,* 1946, v31, 141.

4. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition,* 1937, v14, 273.

TABLE I. Incidence of Hepatic Damage in Choline and Cystine Deficiency.

Initial wt, g	Sacrificed, No.	Died, No.	Time on diet, days	wt change, g	Liver		
					Necrosis, No.	Fibrosis, No.	Fatty, No.
Choline deficient							
50		6	112	+ 8	2	4	4
50	3		270	+ 61	0	3	3
150	5		181	+ 12	0	2	3
150		3	209	— 49	0	2	3
150	6		240	— 24	0	3	6
300		9	165	—118	3	2	6
300	10		273	— 78	0	4	9
Cystine deficient							
50		10	51	— 20	5	0	0
150	6		56	— 57	0	0	0
150		9	91	— 80	3	0	0
300	6		75	—115	0	0	0
300	1	12	143	—184	5	0	0
Choline and cystine deficient							
50		10	56	— 22	0	0	0
150	6		74	— 76	0	0	3
150		8	125	— 88	5	1	2
300	6		75	—127	0	0	3
300		16	170	—180	7	6	5

amination each liver was studied with respect to the incidence and extent of fat accumulation, necrosis and fibrosis. These were graded 0-4+ as described previously(2). The results are summarized in Table I.

Choline Deficiency. In agreement with our previous experience, the response to choline deficiency proved to be quite variable. Of the twelve 50 g rats, 9 died during the experiment but at times varying from 44 to 195 days. Unfortunately 3 of these livers were badly autolyzed and could not be satisfactorily examined. The remaining 6 of the 9 livers in this series showed 1-3+ fibrosis. Two rats showed minimal necrosis and all of those which had gained weight over the experimental period had marked fatty livers as did some, but not all, of those which had lost weight. There was a definite correlation between the length of life and the extent of scarring and fat deposition. Five of the 150 g rats were sacrificed after 18 days. Of these, 3 had extensively fatty livers but showed no necrosis and only 2 evidenced a minimal fibrosis. Of the remaining rats in this group, 66% survived the entire experimental period but all showed fatty livers and half were fibrotic. Like the 50 g rats most of this group gained weight for all but the last month of the experiment and then lost rapidly.

None of the 300 g rats were sacrificed at a time which now permits comparison with younger rats. All of this group lost weight throughout the experiment, yet half of them were alive at 9 months and 15 of the total of 19 had fatty livers. Yet, only 6 showed any fibrosis. Of the total of 28 rats of the two older series which either died of themselves or were sacrificed at the end of the series, only 7 showed a 3+ fibrosis while 17 showed no fibrosis at all despite the fact that 24 of these rats had fatty livers. These findings are in agreement with the impressions gained previously in studies not designed to this end, that hepatic damage due to choline deficiency, beyond the stage of fat accumulation, is much more readily obtained in young than in adolescent or mature rats. The severity of the fibrosis in younger rats was almost entirely dependent upon their survival time but this was not true in the older rats. It is of considerable interest that many of the initially larger animals probably lived for 9 months with massively fatty livers which did not progress beyond this stage. Only under special conditions(3) has this been observed consistently in younger rats.

Cystine Deficiency. In general the relationship observed in choline deficiency also obtained in cystine deficiency, *viz.*, younger rats were much more susceptible than older rats in that they succumbed much sooner. All the rats of the entire series lost between 1/2 and 2/3 of their initial body weight. The mean survival time of the 10 50 g rats was 51 days while, of the older groups allowed to run their course, the survival time of the nine 150 g rats was 91 days and of the twelve 300 g rats, 143 days. Only one rat of the entire series, whose initial weight was 300 g, survived the entire experimental period. In 5 of the 50 g rats, 3 of the 150 g rats and 5 of the 300 g rats were found characteristic necrotic livers. It is noteworthy that, of the 150 g group, the 3 necrotic livers were found in the 3 rats which survived longest.

None of the 13 animals in the 2 older groups, sacrificed at 56 and 75 days respectively, showed any histological abnormalities in their livers. The relatively low incidence of hepatic necrosis among the cystine-deficient animals, despite their uniform survival times and invariable death, raised the problem of the cause of death in the animals with essentially normal livers. A control experiment was run in which 10 rats of each of 3 age groups were fed the basal diet supplemented with both choline and cystine and maintained on this diet for 175 days. At this time, 2 of the 50 g rats and only 1 of each of the other groups had died. Thus the mortality rate on the cystine-supplemented ration was much lower than that in the cystine-deficient series and it must be concluded that early death in the latter group must be ascribed to cystine deficiency even when their livers appear to be essentially normal.

In an attempt to determine the cause of death in those cystine-deficient animals showing no hepatic lesions, another 12 rats weighing 50 g were fed this diet until they died. Sections were made of heart, lung, liver, spleen, pancreas, kidney, adrenal, testis, trachea, thyroid, parathyroid, esophagus, stomach, intestine, eye, brain, skin, bone and marrow. Other than the hepatic necrosis found in half the animals, no lesions were found in any organ. Except for a moderately atrophic skin all tissues were entirely normal in appearance.

Choline and Cystine Deficiency. As in uncomplicated cystine deficiency, all the animals on this diet died during the experimental period. Again, the younger rats were considerably more susceptible than were the older rats, the mean survival times being 56, 125 and 170 days for the 3 age groups, respectively. It is noteworthy that rats deficient in both choline and cystine lived significantly longer than did rats deficient in cystine alone. All rats lost weight throughout the experimental period. The livers of the young rats that died of this dual deficiency were entirely normal in appearance. No explanation is available to account for the protective influence of choline deficiency against the hepatic necrosis of cystine deficiency but it appears likely that the cause of death was similar to that in uncomplicated cystine deficiency. The lack of fatty livers due to the choline deficiency is entirely in accord with all previous experience with choline deficient young rats which lose weight markedly during the experiment. Half of the 150 g rats sacrificed at 74 days had appreciably fatty livers but were neither fibrotic nor necrotic. However, when allowed to progress, half of the remainder developed necrotic livers, presumably due to the cystine deficiency, while only one liver in this group was at all fibrotic. Of the 300 g rats, again half of those sacrificed at 75 days were found to have fatty livers but even these were neither fibrotic nor necrotic. Of the 16 animals allowed to progress, 7 were found to have markedly necrotic livers while 6 were fibrotic. Since only 2 of the livers were both fibrotic and necrotic, it appears likely that these were of different origin, the necrosis having been due to cystine deficiency. The lack of fibrosis in the 150 and 50 g series was probably due to the fact that they did not survive for a sufficient length of time nor had they sufficiently fatty livers. It is of interest that the incidence of fibrosis in the 300 g rats deficient in choline and cystine was of the same order as that in the equivalent choline deficient, cystine fed animals. Most, but not all, of those livers were also fatty, in keeping with the concept that the fibrosis is contingent upon a preliminary fatty period(3). However, it is difficult to understand the maintenance of fatty livers in rats which lost 1/2 to 2/3 of their body weight during the experimental period.

Summary. A study has been made of the variation with age of the response of rats to low casein, cholesterol supplemented diets deficient in cystine and/or choline. Young rats succumb much more readily than do older rats to the effects of dietary deficiencies in cystine or choline. While choline deficiency almost invariably results in hepatic fibrosis in young rats, in older rats it frequently causes nothing but fatty livers even after 8 months. The incidence of hepatic

necrosis due to cystine deficiency in young rats was about the same as that observed in older rats but the young rats developed such lesions in 1/3 the time required in older rats. Many rats of all ages have been observed to die of cystine deficiency with no detectable histological abnormalities in any of 19 organs examined. Young rats, fed a diet deficient in both choline and cystine live somewhat longer than simply cystine deficient animals but show no hepatic damage. Older rats on such rations develop livers which are necrotic, fibrotic, and fatty despite a loss of as much as two-thirds of their initial body weight during the experimental period.

Received June 30, 1950. P.S.E.B.M., 1950, v75.

Blood Levels of I-131 after Tracer Doses in the Diagnosis of Hyperthyroidism. (18269)

SOLOMON SILVER AND MACK H. FIEBER. (Introduced by Paul Klemperer)

From the Physics Laboratory and The Medical Service, Mount Sinai Hospital, New York City.

Up to the present time, all methods employing radioactive iodine in the diagnosis of thyroid function have depended upon the well established fact that the hyperactive thyroid gland selectively concentrates and fixes larger amounts of I-131 than does the normal organ. This phenomenon has been measured in 2 ways; first by direct counting over the gland with a suitably arranged Geiger-Müller tube and second, by measuring the excretion in the urine during a fixed period after the administration of the tagged iodine. Both of these methods leave much to be desired so far as results consistent with the clinical state of the patient are concerned. There is a definite, often considerable, area of overlap between the normals and the hyperthyroids. In an attempt to circumvent these difficulties some observers have attempted to study the blood levels of I-131 after tracer doses and have sought additional information by fractionating the circulating I-131 into its inorganic and protein-bound fractions. These studies have again showed a considerable overlap between the normals and the hyperthyroids and have not given diagnostic information as clear-cut as might be desired. It occurred to us that a new approach might be fruitful. If one attempted to measure *secretion* of the thyroid hormone from the gland into the circulation rather than *fixation* of iodine by the gland more conclusive results might be attained. This has a physiological basis because it is the increased discharge of hormone from the thyroid gland rather than the increased avidity for iodine which accounts for the clinical phenomena of disturbed thyroid function.

Studies reported to date have usually been extended over a period of only 24 hours because it was known that fixation of iodine in the gland is quite complete in that period of time. If, however, one carries the observations over a longer period of time and studies blood levels of I-131, the changes due to fixation in the gland become less significant and changes due to hormone *secretion* dominate the findings. Although there is a difference between the amount of I-131 fixed in the glands of euthyroid and hyperthyroid subjects, these differences are not marked enough for a clear differentiation of the 2 groups of patients. However, at the end of 48, 72 or 96 hours after the administration of the tracer dose of I-131 there is a very marked difference in the level of I-131 in the blood and this difference has diagnostic value. The determination of the radioactivity of 1 cc of blood beyond 24 hours after safe tracer doses of I-131 could not be performed until counters of high sensitivity, such as the flow counters, became available.

Selection of clinical material. A consecutive series of 24 normal and 30 hyperthyroid subjects was selected from the wards of The Mount Sinai Hospital. The normal patients