oil it is evident that the vitamin D content of this oil shows striking variation, which follows the same trend as the vitamin A content in the samples studied (to be discussed in the forthcoming paper by Shorland, 1945).

SUMMARY

1. Prophylactic bone ash analysis was used to determine the vitamin D content of three New Zealand fish oils. The vitamin D content in i.u./g. was found to be: whole-body eel oil, 25; ling liver oil, 260; groper liver oil (I), 5300; groper liver oil (II), 19,000. The potency of the two samples of groper liver oil shows a striking variation, and in the same direction as that of the vitamin A content of these oils.

2. Composite ashing of bones has been shown to yield results as accurate as individual ashing determinations, with rats as experimental animals, as has previously been shown for chickens. This modification of the method is recommended since it greatly reduces the time and labour required.

3. The number of animals required in a group with this particular stock of animals was found to be seven, if the results were to be statistically significant.

I am greatly indebted to Dr Elizabeth Gregory for much helpful criticism and advice; to Dr F. B. Shorland of the Agricultural Department for supplying the ling and groper liver oils; to Mr D. F. Hobbs of the Marine Department, who supplied the whole-body eel oil; and to Dr Muriel Bell and members of the Nutrition Research Department for their assistance, which enabled me to complete this study.

REFERENCES

- Association of Official Agricultural Chemists (1940). *Official and Tentative Methods of Analysis*, 5th ed., p. 371. Washington: Assoc. Off. Agric. Chem. Inc.
- Chick, H., Korenchevsky, V. & Roscoe, M. H. (1926). *Biochem. J.* **20**, 622.
- Coward, K. H. (1938). *The Biological Standardization of the Vitamins*. London: Baillière, Tindall and Cox.
- Cunningham, M. (1935). *N.Z. J. Sci. Tech.* **17**, 563.
- Day, H. G. (1940). *J. Nutrit.* **20**, 157.
- Denz, F. A. & Shorland, F. B. (1934). *N.Z. J. Sci. Tech.* **15**, 327.
- Edisbury, J. R., Lovern, J. A. & Morton, R. A. (1937). *Biochem. J.* **31**, 416.
- Fisher, R. A. (1938). *Statistical Methods for Research Workers*, p. 130. Edinburgh: Oliver and Boyd.
- Griem, W. B. (1939). *J. Ass. off. agric. Chem., Wash.*, **22**, 656.
- Key, K. M. & Morgan, B. G. E. (1932). *Biochem. J.* **26**, 196.
- Kon, S. K. & Henry, K. M. (1935). *Biochem. J.* **29**, 2051.
- Loy, H. W., Jr., DeWitt, J. B. & Knudsen, L. F. (1941). *J. Ass. off. agric. Chem., Wash.*, **24**, 190.
- Motzok, I., Hill, D. C., Brannon, H. D. & Slinger, J. S. (1943). *J. Ass. off. agric. Chem., Wash.*, **26**, 263.
- Oser, B. L. (1939). *J. Ass. off. agric. Chem., Wash.*, **22**, 445.
- Shorland, F. B. (1945). To be published.

The Gross Chemical Changes in the Liver in Dietetic Necrosis

BY H. P. HIMSWORTH AND L. E. GLYNN, *The Medical Unit and the Morbid Anatomy Department, University College Hospital Medical School, London*

(Received 24 April 1945)

Dietetic necrosis of the liver in rats was probably first observed by Weichselbaum (1935) when he noted that rats on a low casein diet developed 'haemorrhages' into the liver. Later macroscopically similar lesions were observed, inconstantly and irregularly, in animals on various deficient diets (György & Goldblatt, 1939; Webster, 1942) and these were proved to be necroses. Recently it has been shown that such lesions can be produced with certainty, that a deficient intake of protein is the single dietary factor correlated with their occurrence, and that diets containing proteins poor in sulphur amino-acids facilitate, while supplements of such amino-acids prevent, the development of the lesion (Himsworth & Glynn, 1944a, b; Himsworth, 1945).

The histological features of the lesion are those of massive acute necrosis of the liver (Glynn & Himsworth, 1944); the gross chemical features are described below.

METHODS

The details of the diets, vitamin supplements and care of the rats have been given elsewhere (Himsworth & Glynn, 1944a). Here it need only be noted that the sources of dietary protein were casein or yeast and that, although the proportions of fat and carbohydrate varied widely in different diets, the protein intake on each was less than 500 mg./rat/day.

White Wistar and black hooded rats were used. At the beginning of experiments, their weights were 90–120 g.; on killing they weighed 50–250 g, depending on the type of

experiment. When results are expressed as g./100 g. body weight the body weight referred to is that at the time of killing.

Liver samples. The animals were killed by a blow on the head and the liver removed, weighed and apportioned as quickly as possible. Livers from animals dying spontaneously were discarded but those from animals moribund from hepatic necrosis were analyzed.

Dietetic necrosis may be generalized or partial. When partial it involves only the left lobes of the liver and, even when generalized, often affects the left lobes preponderantly (Glynn & Himsworth, 1944). At the beginning the right and left groups of lobes were analyzed separately but it was soon found that, despite the often conspicuous differences in appearance, their gross composition was surprisingly similar. Thereafter samples, of the same size, were taken for each estimation from both right and left lobes but analyzed together.

Water. Slices of liver, not more than 2 mm. thick, were dried in tared tubes at 108° for 2 hr., detached from the tube wall, dried for a further 2 hr. at the same temperature, and weighed when cool. Slices of kidney and of the quadriceps femoris muscle were similarly treated.

Fat. This was determined by a modification of the method described by Leathes & Raper (1925) which assays fatty acids and unsaponifiable lipid. The results are expressed as such and not as glyceride, etc.

Nitrogen. The micro-Kjeldahl method was used, after wet incineration, with selenium dioxide as a catalyst. Non-protein nitrogen was estimated in the filtrate obtained by grinding up the liver with sand in a 10% (w/v) solution of trichloroacetic acid (50 ml./g. of fresh tissue). Total nitrogen was estimated on the dried tissue resulting from the determination of the water content. Protein nitrogen was obtained by difference, and the protein content calculated by multiplying this figure by 6.25.

Glycogen. The method described by Murphy & Young (1932) and Evans, Tsai & Young (1931) was used.

Criteria for significance. The result of the estimations of each of the above substances is expressed as a mean, together with its standard deviation. Differences between means were assessed as significant when they exceeded three times the standard error of the difference, which was taken to be the square root of the sum of the squares of the standard errors of the two means concerned.

RESULTS

Wet weight of the liver

The relationship between liver weight and body weight is shown in Fig. 1. This shows that in acute necrosis the liver is disproportionately heavy, but that in animals with healed necrosis the proportionality between liver weight and body weight again returns to normal. It also shows that the range of variation in the weight of the liver at a particular body weight is greater in animals with acute necrosis than in healthy animals or in those with healed lesions. These findings indicate that during the acute stage of necrosis some substance (or substances) accumulates in the liver, and that this accumulation is not subject to those influences

which normally maintain the proportionality between liver weight and body weight within relatively narrow limits.

Fig. 1. Relation of the weight of normal and abnormal livers to body weight.

Water content

The percentages of water and fat in the liver are roughly in reciprocal relation and their sum accounts for approximately three-quarters of the wet weight

Fig. 2. Correlation between necrosis and increase of liver water, and absence of correlation between necrosis and liver fat. S.D. = standard deviation.

of the liver. It is, therefore, only possible to compare the percentage of water in different livers when the percentage of fat in these falls within narrow limits. The mean percentage of water in 24 normal specimens, which contained less than 6% of liver fat, was $70.6\% \pm 1.6$ (where 1.6 is the standard deviation); in 21 necrotic specimens with a similar fat content, $77.6\% \pm 2.8$. The percentage of muscle water in the two groups of animals averaged $75.1\% \pm 1.6$ and $74.3\% \pm 1.6$ respectively; and of kidney water $76.5\% \pm 1.5$ and $77.8\% \pm 1.8$ respectively. The difference between the percentages of liver water in the two groups is significant; that between the percentages of muscle water and of kidney water, insignificant. This point is more clearly brought out when the water content of the liver is expressed as g./100 g. body weight; and this mode of expression has the added advantage that the result is independent of the fat content of the liver (Fig. 2). In 54 normal rats the average amount of liver water per 100 g. of body weight was $3.322 \text{ g.} \pm 0.476$; in 27 rats with acute hepatic necrosis it was $5.288 \text{ g.} \pm 1.070$. These differences are highly significant. It thus appears that in acute hepatic necrosis there is an intrinsic oedema of the liver.

Fat content

The percentage of fat in the liver varies with the composition of the diet. In normal animals no correlation exists between the amount of liver fat and the amounts of other liver constituents which were estimated. This is shown in Fig. 2 where liver fat expressed as g./100 g. body weight is charted against liver water expressed in the same units. It will be seen that in normal animals the liver fat is, as it were, superimposed on liver tissue which in other respects is of relatively constant composition. This observation explains the apparent reciprocity between the proportions of fat and water in the liver. In the case of livers showing acute necrosis there is a similar lack of correlation between the amount of liver fat and the amount of other constituents. Similarly no relationship could be established between the proportion of fat in the liver and the incidence of necrosis. Hepatic necrosis occurred at all levels of liver fat (Fig. 2) and afflicted animals showed the same proportions and amounts of liver fat as normal animals which had been taking the same diet for a similar length of time. Thus in a group of animals on one diet the liver fat content of the 11 with normal livers was $0.219 \text{ g./100 g. body weight} \pm 0.104$, of the 9 with necrotic livers $0.232 \text{ g./100 g. body weight} \pm 0.088$. These observations may appear surprising, first because of the well-known correlation between a high content of liver fat and susceptibility to certain poisons; and second because of the yellow colour of many necrotic livers. Many

have a degree of yellowness which in the normal liver betokens a high proportion of fat; but on analysis no excess of fat is found. The conclusion from these observations appears unequivocal. The development of dietetic necrosis of the liver is unrelated either to the amount or proportion of fat in the liver.

Non-protein nitrogen

Early analyses of livers from fatal cases of chloroform and phosphorus poisoning (Wells, 1907, 1908; Stadie & Van Slyke, 1920) demonstrated the presence of considerable quantities of the products of protein breakdown. If a similar result were found in livers showing dietetic necrosis it might suggest an explanation for their oedema. The liver non-protein nitrogen expressed as a percentage of the total liver nitrogen was, in 21 normal rats, $7.5\% \pm 1.6$; in 20 rats with acute necrosis $8.9\% \pm 3.8$. This difference is hardly significant and would certainly appear insufficient to account for any great increment of liver water. It will be noted that the variation in the values from necrotic livers is greater than in the normals. Actually the values in the necrotic livers ranged from 4.2 to 18.5%. The lower values were obtained from animals killed within a short time of appearing ill; the higher from those who lingered for several days. It is also noteworthy that results on necrotic livers from animals who had been dead for some hours were also high. The indication is, that the high proportion or amount of non-protein nitrogen in a necrotic liver is the result of post-mortem autolysis; it being understood that such autolysis can proceed in dead liver cells before the animal itself dies. It thus seems probable that a significant increase of non-protein nitrogen is not an essential feature of dietetic necrosis of the liver.

Protein

It seemed a reasonable possibility that, massive acute necrosis of the liver being associated with a low intake of protein in the diet, the protein content of necrotic livers would be less than normal; and it was found that the percentage of protein in the liver was decreased. Thus in 21 normal animals the percentage was $17.3\% \pm 2.4$, in 20 animals with necrosis $13.6\% \pm 1.9$. When the same results were expressed as g. liver protein/100 g. of body weight, however, it was found that the amount in the 21 normal animals was $0.761 \text{ g./100 g. body weight} \pm 0.082$, while in the 20 rats with hepatic necrosis it was

* This figure is considerably higher than that reported by Kosterlitz (1944) for rats on the same diets. In his short report the body weight of the rats is not stated but, if the weights were similar to those in previous experiments (Kosterlitz & Cramb, 1943), then his rats were twice or thrice as heavy as ours; and this may account for the higher figure we found in our animals.

0.946 g./100 g. body weight ± 0.121 . These differences are both highly significant. It seems, therefore, that in acute dietetic necrosis of the liver the amount of protein in the liver is increased but that, owing to dilution of the constituents as the result of oedema, the percentage content is decreased.

Glycogen

When the percentages of fat, water and protein in the liver are added together and the resulting sum compared for normal animals and those with hepatic necrosis it is found that the sum in the former is consistently lower than that in the latter. Thus

Table 1. *Data from 20 animals on same diet; 11 normal and 9 necrotic livers*

Average wt. of necrotic livers 6.276 g./100 g. body wt.; of normal livers 4.593 g./100 g. body wt.

Average wt. of liver minus (fat + protein + water) in normal = 0.432 g./100 g. body wt.

Average wt. of liver minus (fat + protein + water) in necrotic = 0.260 g./100 g. body wt.

Difference = 0.172 g./100 g. body wt.

As necrotic livers contain no glycogen this difference can be accounted for by the glycogen content of normal liver.

Average wt. of necrotic livers minus glycogen = 6.276 - 0.000 = 6.276 g./100 g. body wt.

Average wt. of normal livers minus glycogen = 4.593 - 0.172 = 4.421 g./100 g. body wt.

Difference in wt. to be accounted for = 1.855 g./100 g. body wt.

Average difference between fat contents of normal and necrotic livers = (not significant)

Average difference between water contents of normal and necrotic livers = 1.682 g./100 g. body wt.

Average difference between protein contents of normal and necrotic livers = 0.156 g./100 g. body wt.

Total of differences = 1.838 g./100 g. body wt.

Thus the increase in wt. of necrotic over normal livers can be accounted for by the increased protein and water contents of necrotic livers.

in 21 normal livers the sum was $92.4\% \pm 2.5$; in 18 necrotic livers it was $95.6\% \pm 1.2$. This significant difference suggests that something, which normally accounts for about 3% of the liver solids, has disappeared from the livers showing necrosis. The percentage of glycogen in the liver is of this order and it has long been known that in necrosis caused by poisons such as phosphorus, glycogen disappears from the liver (Saikowski, 1865). On analysis it was found that livers with dietetic necrosis contained no trace of glycogen while those from healthy animals on the same diets contained between 1.5 and 4% of glycogen.

Unfortunately analyses for liver glycogen were not carried out on all animals so it is not possible to prove conclusively that the difference between the sums of the amounts of protein, fat and water in normal and necrotic livers is accounted for by glycogen. But the evidence available suggests that it is. Necrotic livers contain no glycogen and in nine animals the difference between the liver weight and the sum of the protein, fat and water, all expressed as g./100 g. of body weight, averages 0.260 ± 0.053 . In the few normal in which glycogen was estimated the difference between the total liver weight and the sum of the glycogen, protein, fat and water falls within this range.

DISCUSSION

The differences between necrotic and normal livers revealed by the preceding results are that necrotic livers contain more water, more protein and no glycogen. No significant differences were demonstrated between the amounts of fat and of unanalyzed residue and between the proportions of non-protein nitrogen, in the two types of liver. To test whether the difference between the wet weights of necrotic and normal livers can be accounted for by the observed changes in composition a balance sheet has been drawn up. In Table 1 it can be seen

that the increased weight of necrotic livers is satisfactorily explained by the increase in their water and protein contents; that is by the uptake of a protein solution of a concentration more dilute than that of normal liver tissue. From the data in the table it appears that, for this particular group of necrotic livers, such a protein solution would have a concentration of 9.3 g./100 ml. This is considerably stronger than that of rats' plasma; but there is collateral evidence that plasma contributes to it. In rats ill from dietetic necrosis the haemoglobin concentration of the circulating blood is increased by about 20% above the level for animals on the same diet. As death approaches, the animals pass into a state of 'shock' which clinically resembles that of patients with a low plasma volume. The source of the extra protein, above that which can be accounted for by absorbed plasma, is uncertain. The histological appearances do not suggest that it is derived from infiltrating inflammatory cells or by packing of red blood corpuscles into dilated liver sinusoids. A similar accumulation of protein and water was reported by Hoppe-Seyler (1921), Never (1932), and Uher (1939) in tissues showing the milder degeneration of 'cloudy swelling'; and we have noted in partial hepatic necrosis that the lobes which are grossly normal, and show

only slight histological changes, have a similar composition to the adjoining necrotic tissue. In neither of these instances does cellular infiltration occur. It seems, therefore, that the chemical changes observed indicate an actual alteration in the composition of the liver protoplasm. It also appears that these changes are qualitatively not characteristic of established necrosis but occur, at least to some extent, at the inception of cellular damage.

A remarkable feature of these alterations is that they appear suddenly. Where hepatic necrosis appears in a few animals of a group, then, if the whole group is killed, it is found that the livers are either necrotic and of abnormal composition, or normal both in appearance and composition. There is no transition between the two, nor any evidence of a gradual alteration in composition leading steadily to necrosis. It thus seems that at a particular time after exposure to appropriate dietetic conditions some sudden derangement of metabolism occurs in the liver; and this leads to necrosis and the observed

alterations in liver chemistry. The question whether these alterations are the result or the cause of cellular damage is of minor importance for in either case they must be secondary to that sudden derangement of metabolism in the liver cells.

SUMMARY

1. Livers affected by acute dietetic necrosis show, as compared with livers from normal rats, increased amounts of water and protein and an absence of glycogen. There is no increase in non-protein nitrogen, and no relationship between the fat content of the liver and the development of necrosis.

2. Livers with healed necrotic lesions do not show these abnormalities.

3. The increased weight of necrotic livers is satisfactorily accounted for by their increased content of water and protein, and some, at least, of the extra protein and water seems to be derived from plasma.

4. The alterations in composition appear suddenly with the onset of cellular damage.

REFERENCES

- Evans, C. L., Tsai, C. & Young, F. G. (1931). *J. Physiol.* **73**, 67.
 Glynn, L. E. & Himsworth, H. P. (1944). *J. Path. Bact.* **56**, 297.
 György, P. & Goldblatt, H. (1939). *J. exp. Med.* **70**, 185.
 Himsworth, H. P. (1945). *Proc. roy. Soc. Med.* **38**, 101.
 Himsworth, H. P. & Glynn, L. E. (1944a). *Clin. Sci.* **5**, 93.
 Himsworth, H. P. & Glynn, L. E. (1944b). *Clin. Sci.* **5**, 133.
 Hoppe-Seyler, G. (1921). *Hoppe-Seyl. Z.* **116**, 67.
 Kosterlitz, H. W. (1944). *Nature, Lond.*, **154**, 207.
 Kosterlitz, H. W. & Cramb, I. D. (1943). *J. Physiol.* **102**, 18P.
 Leathes, J. B. & Raper, H. S. (1925). *The Fats*. London: Longmans, Green and Co.
 Murphy, G. E. & Young, F. G. (1932). *J. Physiol.* **76**, 395.
 Never, H. E. (1932). *Zbl. allg. Path. path. Anat.* **54**, 327.
 Saikowski (1865). *Virchows Arch.* **34**, 73.
 Stadie, W. C. & Van Slyke, D. D. (1920). *Arch. intern. Med.* **25**, 693.
 Uher, V. (1939). *Beitr. path. Anat.* **102**, 544.
 Webster, G. I. (1942). *J. clin. Invest.* **21**, 385.
 Weichselbaum, T. E. (1935). *Quart. J. exp. Physiol.* **25**, 363.
 Wells, H. G. (1907). *J. biol. Chem.* **3**, Proc. xv.
 Wells, H. G. (1908). *J. biol. Chem.* **5**, 129.

Observations on the Nature of Vitamin P and the Vitamin P Potency of Certain Foodstuffs

By H. SCARBOROUGH, *The Clinical Laboratories, The Royal Infirmary, Edinburgh*

(Received 26 April 1945)

The material first described by Szent-Györgyi and his co-workers (Armentano, Bentsáth, Beres, Rusznyák & Szent-Györgyi, 1936) as increasing capillary resistance in man was called by them citrin or vitamin P. Subsequent statements (e.g. Szent-Györgyi, 1937) that citrin is a mixture of the flavanone glycosides hesperidin and the more soluble and more active eriodictyol glycoside (called eriodictin) have not, so far as we know, been supported by any published evidence. The chemical nature of many active extracts is unknown, but vitamin P activity has been attributed variously to flavones, flavanones and flavanols.

Mager (1942) attempted a chromatographic analysis of citrin and isolated a faintly yellow crystalline product (m.p. 184–186°) which was identified as eriodictyol rhamnoside. Crystalline eriodictyol was obtained by hydrolysis (m.p. 258–60°). There is no report of any biological test having been carried out on this material, which was readily soluble in water, gave an intense cyanidin reaction and a transient green colour with ethanolic FeCl₃. Previous chromatographic studies by Robeznieks (1938) had indicated the presence in citrin of an unidentified flavonolglycoside similar to quercetin, in addition to hesperidin and eriodictin. According to Bentsáth,