BRITISH MEDICAL JOURNAL

LONDON SATURDAY JANUARY 11 1958

FATTY LIVER OF ENDOCRINE ORIGIN

WITH SPECIAL REFERENCE TO FATTY LIVER OF MALNOURISHED AFRICAN INFANTS

BY

JOSEPH GILLMAN, D.Sc., M.B.

AND

CHRISTINE GILBERT, D.Sc.

Departments of Physiology and Anatomy, and the Joint Nutrition Research Unit of the C.S.I.R., and of the University of the Witwatersrand, Johannesburg

Fatty liver is one of the constant features of African infants suffering from pellagra and kwashiorkor (Gillman and Gillman, 1945). A mildly fatty liver containing approximately 8% of total lipids early in the illness may evolve into one containing over 40% of total lipids within nine days (Gillman and Gillman, 1951). Such a rapid evolution of the fatty change suggested that the acute episode in infants was the result of a sudden metabolic disorder occurring against a background of chronic malnutrition. This view implies that there are two stages in the natural history of florid kwashiorkor and infantile pellagra. The first, determined by chronic malnutrition, is initiated before or soon after weaning. No fatty liver develops during this stage, but the reactivity of the infant is altered, with the result that a mild intercurrent infection, a sudden change in temperature, or a mild gastro-intestinal upset may precipitate the second stage, which declares itself as florid kwashiorkor or as infantile pellagra. During the second stage a fatty liver develops rapidly. According to Davies (1948) and Trowell *et al.* (1954), this fatty liver is possibly due to "an almost complete suppression of the pancreatic enzymes from the gut."

The present experimental study was undertaken to ascertain whether a fatty liver of endocrine origin to be described in the baboon was in any way relevant to the mechanism concerned with the rapid development of a fatty liver of infants suffering from a florid form of infantile pellagra or kwashiorkor. It will be our purpose (1) to emphasize the importance of insulin, cortisone, and also of thyroxine in the pathogenesis of hepatic steatosis; (2) to demonstrate that in the presence of adequate thyroxine the mobilization of fat, even when excessive, will not result in a fatty liver; and (3) to suggest that the fatty liver of malnourished African infants is dependent not so much on the atrophy of the exocrine part of the pancreas as on the emergence of a particular endocrine physiology, the overall effect being a relative adrenocortical hyperactivity, accompanied by a relative or absolute depression of insulin.

Material and Methods

Twelve male and 17 female baboons were used in the present investigation. Nine male baboons were totally pancreatectomized, and thereafter the diabetes was controlled with long-acting insulin (Boots N.P.H.) which was administered twice daily, in the early morning and

late afternoon. The morning urine sample was tested daily for its sugar content and twice weekly a check was kept on the resting blood sugar. In this manner the insulin requirements of each baboon were established. The maintenance dose of insulin ranged from 24 to 48 units daily and was not related proportionally to the body weight of the baboon. Four of the depancreatized male baboons were totally hypophysectomized at intervals of 4 to 15 months after removal of the pancreas, the operation being performed according to the method of Dr. E. Epstein (to be published).

Three male and 10 female baboons were totally thyroidectomized. An additional five female baboons were hypophysectomized, while in the remaining two the stalk of the pituitary gland was sectioned. The latter was effected by inserting a platinum plate between the pituitary and the diaphragma sellae, thereby excluding any possibility of a vascular connexion being re-established between the pituitary and the hypothalamus.

Cortisone was administered on a body weight basis—namely, 1 mg./kg. body weight/day, the injection being given in two divided doses. Except where stated otherwise, the dosage of thyroxine was the same for all the baboons—namely, 0.5 mg. three times weekly.

Experimental

Hypophysectomy.—Fat failed to develop in the livers of any of the four baboons during an eight-months period following hypophysectomy.

Thyroidectomy.—Complete thyroidectomy of male and female baboons was not associated with the development of a fatty liver even when observed over a four-year period. In this respect the baboon behaved differently from the dog (Chaikoff *et al.*, 1948).

Pancreatectomy and Fatty Liver

Total pancreatectomy was performed on nine male baboons ranging in age from 3 to 5 years. The small fragments of pancreas which usually adhere closely to the duct and extend into the wall of the duodenum were carefully dissected away, but the duodenum itself was left intact. Within six hours after the operation the blood sugar rose to between 200 and 350 mg. per 100 ml., and the baboons became dependent on insulin for the control of the diabetes. Appetite, and the preferences displayed by some baboons for vegetables, and by others for the prepared ration (Gillman and Gilbert, 1956f), influenced, but did not alone determine, the insulin requirements of the individual baboon.

Experience disclosed that if fatalities were to be avoided it was undesirable to cause the blood sugar to fall too low during the night. Only a small amount of long-acting insulin was administered in the evening, with the result that in all the pancreatectomized baboons the morning sample of urine contained sugar, while the resting blood sugar was raised to between 170 and 400 mg. per 100 ml. Although the diabetes was controlled during the day, it was not entirely controlled for a period of approximately 8 to 10 hours during the night. If these facts are borne in mind, it is now possible to examine the effects of pancreatectomy on the fat content of the liver.

Under the conditions of our experiments, some fat is frequently, but not invariably, found in the livers of insulin-treated, depancreatized baboons. The amount of fat in the liver is a function of the efficiency of the control of the diabetes rather than of the lack of some pancreatic factor other than insulin, as seems to be the case in the dog. Where we have attempted to control the diabetes effectively by giving increased amounts of insulin during the day and night, histologically visible fat can be removed completely from the liver. Even where the diabetes is less well controlled and fine droplets of fat are present in the cells of the periportal region, the total fat content of the liver does not exceed 8%. The latter represents only a small increase above control values and cannot be compared with the much greater accumulation of the fat reported by Prohaska *et al.* (1936), Dragstedt *et al.* (1936), and Chaikoff *et al.* (1947) in insulin-treated depancreatized dogs.

Lipocaic, free choline, methionine, or pancreatic enzymes are apparently not required by the depancreatized baboon as they are by the dog. Possibly the gut of the baboon elaborates a substance akin to lipocaic or may compensate for the loss of pancreatic enzymes by some other mechanism still to be elucidated. Length of survival after pancreatectomy does not influence the fat content of the liver. Baboons surviving for 18 months do not show any progressive increase in liver fat. These results disclose that, so far as the baboon is concerned, the exocrine part of the pancreas does not influence appreciably the presence or absence of fat in the liver.

The withdrawal of insulin from depancreatized baboons is followed sooner or later by grave constitutional signs, although most animals can tolerate an insulin-free period of four to five days. Without exception, the fat content of the liver increases rapidly in every baboon after withdrawal of insulin. Chemical estimations disclosed a fat content of over 40% of the wet weight of the liver.

Within 48 hours after the withdrawal of insulin from depancreatized baboons the liver cell fills rapidly with discrete globules of fat which are distributed throughout the lobule. Although at first the fat seems to be more abundant in the periportal region than elsewhere, this localization becomes less apparent as fat accumulates in all the cells of the lobule. A striking and consistent feature is the discrete nature of the fat globules which fill the enlarged cell. The globules may later fuse to form a single large fat droplet occupying the greater part of the cytoplasm, emphasizing the similarity of structure between the fatty liver of malnourished infants and that of the insulin-deprived depancreatized baboon. As the fat accumulates in the baboon's liver the glycogen disappears until eventually only histochemically detectable glycogen is to be found in the large, vacuolated nuclei.

As soon as insulin therapy is begun the lipaemia, ketosis, and hyperglycaemia subside rapidly—that is, within four to six days—while two weeks may elapse before the liver fat is reduced to levels comparable to those prevailing prior to the withdrawal of insulin. The fat seems to disappear first from the mid-zone, and for a while remains localized to the central and peripheral parts of the lobule. Later the fat disappears for the most part from the central zone and is then confined to the periportal region for several weeks, the precise time depending on the degree of control of the diabetes. The remarkable accumulation of fat in the liver

of the insulin-deprived depancreatized baboon and the reduction in fat after the control of the diabetes emphasize the crucial role of insulin in regulating the amount of fat in the liver of depancreatized baboons.

Effect of Hypophysectomy on Fatty Liver of Depancreatized Baboons

Since the absence of insulin from depancreatized baboons promotes the rapid movement of large amounts of fat into the liver, it remained to be determined whether this movement of the fat was due to the unantagonized effect of other hormones, the secretion of which was immediately regulated by the pituitary gland, or was entirely a function of insulin alone.

The pituitary gland was removed completely from four depancreatized baboons. The animals remained in good condition after the operation, and one animal showed marked clinical improvement after hypophysectomy. In all cases the insulin requirements were reduced to one-third or one-quarter of the prehypophysectomy levels.

When deprived of insulin, the blood sugar of the depancreatized-hypophysectomized (D.-H.) baboons continues to rise and the glycosuria becomes intense, the urine containing over 2 g. of sugar per 100 ml. The severe lipaemia and the ketosis are prevented, but the phospholipids and the total cholesterol may continue to rise to near prehypophysectomy levels. It is thus evident that, while the disorder of carbohydrate metabolism has not been alleviated in depancreatized baboons after removal of the pituitary, the rise in the triglyceride component of the blood fats has been suppressed (Gillman *et al.*, 1957a).

The small amount of fat present in the liver of the depancreatized insulin-treated baboon may persist after hypophysectomy and for as long as insulin is administered. Insulin withdrawal, however, is not followed by the accumulation of fat in the liver, and the little fat present before withdrawal may disappear completely. In other words, the fatty liver fails to develop in insulin-deprived D.-H. baboons even at the end of nine days. During this prolonged period of insulin withdrawal the baboons remain clinically well, in contrast to depancreatized baboons, which, as mentioned above, may show marked constitutional reactions if deprived of insulin for four to five days.

These findings in the four D.-H. baboons indicated that the fatty liver of the depancreatized baboon was due not only to a lack of insulin but also to the activity of other endocrine glands immediately regulated by the hypophysis. An opportunity thus became available for assessing the importance of the adrenal gland and of the thyroid in the genesis of a fatty liver.

Effect of Cortisone and of Thyroxine on Liver Fat in D.-H. Baboons

Two D.-H. baboons on insulin therapy received a 23-day course of treatment with cortisone. Withdrawal of insulin during the period of cortisone treatment was associated with a return of the intense lipaemia, but was unaccompanied by ketosis. The liver became fatty once again, although the estimated amount of fat was approximately half that observed in the same animal during an insulin-withdrawal period before hypophysectomy. The administration of insulin to the D.-H. baboons receiving cortisone promptly led to resorption of the fat. If, thereafter, thyroxine was injected every second day in conjunction with cortisone for 15 days, the withdrawal of insulin was rapidly followed by a fatty liver as intense as that observed in the insulin-deprived depancreatized baboon.

The administration of thyroxine alone for 21 days to two D.-H. baboons did not produce a fatty liver in the absence of insulin.

Two insulin-treated D.-H. baboons received cortisone for 53 days, and then thyroxine as well for a further 32 days. The cortisone and thyroxine were withdrawn, but the insulin was continued for another 14 days. Thereafter the

insulin treatment was stopped for six days, at which time another liver biopsy was taken. The histological section revealed the presence of abundant liver fat, indicating that even 14 days after withdrawal cortisone was still active in the baboon.

In short, these experiments in the D.-H. baboons indicated that, in the absence of insulin, cortisone alone could promote a moderately fatty liver which could be intensified by thyroxine, leading to a picture indistinguishable from the liver of a depancreatized baboon deprived of insulin. Cortisone continues to assert itself in the baboon at least 14 days after the last injection.

We should add that we failed to produce a fatty liver in a single depancreatized baboon receiving insulin after the administration of bovine growth hormone in amounts shown by Campbell *et al.* (1953) to produce an intensely fatty liver in dogs. In view of the controversy about the species specificity of growing hormone, we hesitate to attach any importance to a single observation in the baboon. But our experiments disclose that at least one kind of fatty liver can be produced without growth hormone or adipokinin.

Effect of Cortisone, Thyroxine, and Cortisone and Thyroxine in Hypophysectomized and Stalk-sectioned Baboons

Cortisone

Five hypophysectomized female baboons and two stalk-sectioned baboons received cortisone daily for 26 to 180 days. Serial biopsies of the liver disclosed that cortisone treatment for 56 days was unassociated with the development of any fat in the liver. When continued for 93 days the liver became moderately fatty, large globules of fat occupying the cells of the outer half of the lobule, and much smaller droplets the central region. At the end of 180 days the liver was intensely fatty and resembled that of the depancreatized baboons not receiving insulin.

Prolonged treatment with cortisone also resulted in a progressive increase in round cells and a substantial thickening of the portal tracts as well as of the reticulin of the related sinusoids. Moreover, in one hypophysectomized baboon an occasional vacuolated nucleus, similar to those described in depancreatized insulin-deprived baboons, was observed in the liver cells bordering the portal tracts. The blood sugar was not elevated in the cortisone-treated hypophysectomized baboons, although the resistance to insulin was increased and there was evidence of some intolerance to glucose as reflected by the intravenous glucose test (unpublished data).

As we were interested in defining the conditions promoting oestrogen-withdrawal bleeding in hypophysectomized baboons (Gilbert and Gillman, 1956, 1957), we had occasion to pretreat two baboons with cortisone for 26 and 44 days respectively, and then to subject them to a further course of cortisone in conjunction with oestrogen (0.1 mg. of oestradiol benzoate daily), the oestrogen being withdrawn after 14 days but the cortisone continued. Pretreatment with cortisone, followed by simultaneous injections of oestrogen and cortisone, led to the accumulation of fine droplets of fat throughout the lobule, with coarse globules concentrated in the liver cells in the periportal sector. The course of treatment with oestradiol benzoate thus appeared to accelerate the accumulation of fat in the liver of hypophysectomized baboons receiving cortisone therapy.

In this connexion it should be mentioned that a course of treatment with corticotrophin (1 unit/kg. body weight/day) for 46 days, followed by corticotrophin and oestradiol benzoate for 13 days, resulted in a fatty liver even more severe than that produced by cortisone and oestradiol benzoate mentioned above. Again the fat was concentrated chiefly in cells of the outer half of the lobule.

It is thus apparent that cortisone alone, in the concentrations used, will promote a fatty liver in hypophysectomized baboons if treatment is continued for longer than 93 days.

If the fat in the liver is derived from the depots, then the liver can cope with the increased load for an extended period of time. Moreover, the speed of development of the fatty liver in hypophysectomized baboons receiving cortisone or corticotrophin can be accelerated by the administration of oestradiol benzoate.

Two baboons which had received daily cortisone therapy for 126 and 200 days respectively and showed an intensely fatty liver on biopsy were thereafter subjected to a 14-day course of treatment with thyroxine in conjunction with the cortisone. At the end of 14 days the fat content of the liver was sharply reduced. During this short period of time, therefore, thyroxine initiated a rapid decline in the fat content of the liver.

Thyroxine

Thyroxine (0.2 mg./2nd day) was administered to three hypophysectomized baboons and to one stalk-sectioned baboon for 21 to 28 days respectively. All the animals lost weight, and, in view of the severe constitutional disturbances in the hypophysectomized baboons, the treatment could not be continued for a longer period. Fine particles of fat were found in the cells throughout the liver lobule, but, unlike the cortisone-treated hypophysectomized animals, the fat was localized almost exclusively in the sinusoidal pole of the cell.

Cortisone and Thyroxine

Three hypophysectomized baboons and two stalk-sectioned baboons received cortisone daily for a minimum of 56 days, followed by the simultaneous administration of cortisone and thyroxine for a further 38 days. Cortisone treatment was therefore administered uninterruptedly for a minimum period of 94 days. Biopsy of the livers of the baboons at the end of treatment with both hormones showed the presence of fine fat particles in the liver cells throughout the lobule.

The simultaneous withdrawal of cortisone and thyroxine was followed by a steady increase in the fat content of the liver cells. By the 13th day the fat particles were larger, and by the 27th day had coalesced to form large globules, completely filling the cell. The liver then presented a picture indistinguishable from the severely fatty liver of malnourished infants. In fact, this type of fatty liver was more severe than any other thus far produced experimentally in baboons.

Death supervened in three animals within 25 to 99 days after the withdrawal of cortisone and thyroxine.

It should be mentioned that during an earlier experiment one baboon received a 41-day course of treatment with cortisone followed by 38 days' treatment with cortisone and thyroxine. The total period of cortisone treatment was therefore 79 days. The simultaneous withdrawal of cortisone and thyroxine in this baboon was not followed by the development of a fatty liver even at the end of 45 days. On a subsequent occasion, when the baboon had received cortisone uninterruptedly for 162 days, including the terminal 36 days of therapy with cortisone as well as thyroxine, the simultaneous withdrawal of both hormones was followed by the development of a severely fatty liver and death within 26 days. These two experiments in the same baboon indicate that the total length of treatment with cortisone is important for the production of an intensely fatty liver following the withdrawal of cortisone and thyroxine. The fatal outcome of this kind of fatty liver, despite all kinds of treatment, including the intragastric feeding with protein-enriched diets, is to be stressed, and is reminiscent of that which obtains in malnourished infants also suffering from a fatty liver of similar intensity. Not the malnutrition but the severe metabolic disorder is immediately responsible for death.

Discussion

Depancreatized baboons deprived of insulin for several days, like other mammals in similar circumstances, accumulate large amounts of fat in the liver. In cats

(Long and Lukens, 1936), as in baboons, this kind of fatty liver can be prevented by the removal of the hypophysis. Since cortisone promotes a fatty liver in insulin-deprived D.-H. baboons, and since Long and Lukens have shown that the fatty liver fails to emerge in depancreatized, adrenalectomized, insulin-deprived cats, but does so after the injection of cortical extracts, it follows that in depancreatized cats and baboons deprived of insulin the pituitary, via its action on the adrenal cortex, is essential for the production of the fatty liver. In the case of D.-H. baboons, thyroxine potentiates the fat-promoting effect of cortisone. Evidently the fatty liver of depancreatized baboons is due not only to the lack of insulin but also to the presence of an adrenocortical hormone akin to cortisone and in amounts greater than that available in hypophysectomized baboons.

In the presence of adrenocortical activity and in the absence of insulin, the intensity of the fatty change in the liver is regulated by thyroxine. The fatty liver then is dependent on a dynamic relationship between insulin, adrenocortical hormone, and thyroxine. If this relationship is disturbed, such that insulin is relatively or absolutely decreased beyond a level still to be defined, fat will accumulate in the liver, whereas, if insulin is relatively increased, the fatty liver will be prevented. It is significant that the insulin requirements of D.-H. baboons are greater after cortisone therapy, and the fatty liver will be prevented only if the amount of insulin is increased.

Even in hypophysectomized or stalk-sectioned baboons the administration of sufficient cortisone for at least 90 days will so alter the insulin-adrenocortical relation that the liver will become moderately fatty. With the appearance of fat in the liver the blood lipids may also rise. These two events, taken in conjunction with the presence of occasional vacuolated nuclei in the liver cells near the portal tracts, of the kind previously reported in depancreatized baboons (Gillman and Gilbert, 1956a), more than suggest a relative or absolute deficiency of insulin.

During the process of continued fat synthesis and storage and with the expansion of the fat depots, consequent on prolonged treatment with cortisone, the baboon develops a measure of intolerance to glucose, acquired perhaps through the elaboration of an insulin inhibitor similar to that described by Bornstein (1953), or through the production of an alloxan-like substance (Loubatières, 1954). There is, however, no glycosuria, and the resting blood sugar is either normal or at most reaches the upper limits of normality.

It is unlikely that the pancreas is slowly exhausted of its insulin by cortisone, because the administration of large amounts of thyroxine to cortisone-treated hypophysectomized or stalk-sectioned baboons leads to a reduction in the blood lipid, mobilization, and oxidation of the depot fats with a sharp decrease in body weight. If the islets were exhausted, this increased metabolism, consequent on thyroxine administration, should still further emphasize the insulin deficit, but this does not happen. The cortisone-produced fatty liver of the hypophysectomized baboon, therefore, also seems to depend on an altered insulin-cortisone relationship.

The fatty liver consequent on cortisone-thyroxine withdrawal develops consistently in hypophysectomized or stalk-sectioned baboons treated with cortisone for a period of not less than 90 days, including the terminal 38 days during which thyroxine is also administered. Within 25 days after withdrawal of both hormones the liver is extremely fatty. Since little if any fat is present in the liver on the day of withdrawal of both hormones, even in circumstances where cortisone has been injected for 162 days and thyroxine for the terminal 60 days of the experiment, it is evident that thyroxine prevents the deposition of fat in the liver.

Significant, too, in this connexion is the observation that so long as thyroxine is administered the protein-bound iodine (P.B.I.) is raised, but soon after withdrawal of cortisone and thyroxine the P.B.I. drops rapidly to the very

low levels found in hypophysectomized baboons (van Zyl, 1955). This reduction in P.B.I. coincides with the arrest of weight loss, and within a few days the fat accumulates rapidly in the liver.

The fatty liver of cortisone-thyroxine withdrawal therefore begins to emerge when the thyroxine has been metabolized. Since the effects of cortisone certainly persist in D.-H. baboons for 14 days and in hypophysectomized baboons for at least six to eight weeks after the last treatment, the fatty liver of cortisone-thyroxine withdrawal evidently develops when the amount of thyroxine is steadily decreasing and when cortisone is still asserting itself.

As long as thyroxine is available in adequate amounts this kind of fatty liver is prevented. It would appear that thyroxine not only antagonizes the effect of cortisone but also facilitates the mobilization of fat, which in turn is oxidized. Furthermore, thyroxine improves the utilization of carbohydrate in hypophysectomized or stalk-sectioned baboons receiving prolonged treatment with cortisone (unpublished data), thus revealing that the intolerance to carbohydrate induced by cortisone depends in some way on active fat synthesis and fat storage and on a different participation of insulin in metabolism. It may well be the case that thyroxine prevents the elaboration of Bornstein's metabolite which inhibits insulin or of Loubatières's alloxan-like substance which depresses the islets.

On the other hand, cortisone protects the hypophysectomized baboon from the grave constitutional reactions of thyroxine. It is tempting to ascribe this protective effect of cortisone to its role in fat metabolism, especially as it has been demonstrated that the feeding of fat can protect rats against the growth-inhibiting and lethal effects of excess thyroxine (Deuel, 1954).

The commanding role of thyroxine in preventing the cortisone fatty liver is demonstrated by the fact that the fatty livers of hypophysectomized or stalk-sectioned baboons treated for at least 90 days with cortisone will resolve almost completely within 21 days after the thrice-weekly injection of 0.5 mg. of thyroxine. We have reason for believing that thyroxine may be of some clinical value in treating the fatty livers which often appear in patients suffering from Cushing's disease or which develop in the course of chronic alcoholism.

In essence, then, the cortisone-thyroxine withdrawal fatty liver is no different from that produced after the prolonged administration of cortisone, except that in the former instance the liver is much more fatty and the speed of accumulation of fat in the liver is greatly accelerated. Both depend on the presence of excess cortisone, and both are associated with an intolerance to carbohydrate, suggesting a relative insufficiency of insulin. However, there can be little doubt that the sudden removal of thyroxine, as well as of cortisone, promotes a violent change in metabolism from one in which the oxidation of fat and carbohydrates is proceeding vigorously to one in which the utilization of carbohydrate is depressed. The conditions are thus created for the emergence of the intensely fatty liver which develops so characteristically in hypophysectomized and stalk-sectioned baboons.

Less easy to understand is the moderately fatty liver of hypophysectomized baboons receiving excess thyroxine. The grave constitutional reactions attended by the loss of appetite and consequent inanition could easily depress the pancreas. But, since the fatty liver also requires cortical hormone apparently in excess of that elaborated by the hypophysectomized baboon, it might be assumed that the thyroid stimulated adrenocortical activity. This explanation is unlikely, as we have demonstrated that thyroxine does not lead to a fatty liver in D.-H. insulin-deprived baboons. This kind of fatty liver still remains to be explained.

Relation of Liver Fat to Depot Fat

The remarkable lipaemia of depancreatized insulin-deprived baboons, and the expansion of the fat depots of cortisone-treated hypophysectomized baboons (Gillman

et al., 1957), more than suggests that the fat in the liver is closely linked to the factors which regulate the synthesis, oxidation, storage, and mobilization of depot fat. Barrett *et al.* (1938) and Stetten and Salcedo (1944) have demonstrated with the aid of deuterium that fat accumulating in the livers of rats treated with extracts of the anterior pituitary gland is mobilized from the depots. Tracer studies using ^{14}C have revealed that in insulin-deprived depancreatized baboons the fat is also probably mobilized into the liver from the depots (unpublished data).

Mobilization of fat into the liver, according to Levin and Farber (1951), involves two processes. First, the fat-mobilizing mechanism is sensitized by some fraction of the pituitary which apparently is neither growth hormone nor corticotrophin. Once this sensitization has been achieved the second process is initiated by the administration of corticotrophin or 11-oxy corticosteroids.

The experiments in D.-H. baboons disclose that in the absence of the pituitary the administration of cortisone will promote a fatty liver provided the insulin is inadequate or no insulin is supplied to the animal. If at the same time thyroxine is also administered the liver will accumulate massive amounts of fat. These experiments allow us to infer that a fatty liver can be produced in the absence of the pituitary gland in baboons, and that the dynamic relation, particularly between insulin and cortisone and also to a less extent between these two hormones and thyroxine, determines the presence or absence of fat in the liver and therefore also the storage and mobilization of fat from the depots. It would also appear that the preliminary sensitization process required by Levin and Farber before cortisone or corticotrophin can mobilize the fat may represent nothing more than depression of insulin secretion or a sudden change in one of the metabolic actions of insulin, where the insulin becomes insufficient to cope adequately with the oxidation of carbohydrates, thus creating an essential condition for the 11-oxy corticosteroids to mobilize the depot fat to the liver, an effect which then is potentiated by thyroxine.

The fatty liver following the administration of growth hormone is apparently also due to an absolute or relative insufficiency of insulin. Thus, Campbell *et al.* (1954) reasoned, "these observations on liver lipids lead again to the conclusion that growth hormone creates an increased metabolic demand for insulin which is countered in the intact animal by an increase in the rate of secretion of insulin by the islet cells." However, it is to be emphasized that a relative or absolute insufficiency of insulin alone will not produce a fatty liver unless there is also available a minimum amount of 11-oxy corticosteroids.

Rapid mobilization of fat from the depots alone need not lead to a fatty liver. We have indicated that the mobilization of depot fat by thyroxine in a stalk-sectioned or hypophysectomized baboon, rendered obese by prolonged cortisone treatment, is accompanied by a reduction in liver fat, despite the loss of weight. Amongst their other metabolic effects, therefore, insulin and cortisone stimulate fat synthesis and storage in the depots, while insulin and excess thyroxine promote fat mobilization and fat oxidation. Even if fat synthesis also occurs in the presence of thyroxine, as suggested by Wertheimer and Shapiro (1948), the net effect, so far as baboons are concerned, and having regard to the concentration of thyroxine used, is depletion of the fat depots and loss of body weight (Gillman and Gilbert, 1956b).

As reported previously, depletion of the fat depots after adrenalectomy of the baboon is not accompanied by the appearance of fat in the liver (Gillman and Gilbert, 1956c). Synthesis of fat and storage in the depots appears to require a minimal amount of cortical hormone as well as insulin, since if the latter is not available the fat is rapidly moved out of the depots into the liver. The synthesis, storage, mobilization, and oxidation of fat, therefore, are governed by a delicate interaction at least of insulin, cortical hormone, and thyroid hormone. The tendency towards fat synthesis following an increase of cortisone, for example,

may be offset by a corresponding increase in thyroxine. The fat-mobilizing effect of insulin insufficiency may be counteracted by a depression in cortical hormone.

The promotion of the fatty liver by growth hormone, too, needs to be examined in terms of the dynamic relation existing between insulin, thyroxine and cortical hormone. In reviews of the literature, Young (1953) and Engel (1955) outlined the main effects of growth hormone on metabolism. These effects include the inhibition of the hexokinase reaction (Colowick *et al.*, 1947), stimulation of the conversion of amino-acids to protein, and inhibition of amino-acid catabolism (Russell and Cappelletto, 1949; Russell, 1955), mobilization of fat from the depots to the liver (Szego and White, 1949; Greenbaum and McLean, 1953a), acceleration of fatty acid catabolism (Greenbaum and McLean, 1953b), and inhibition of oxidation of acetate via the Krebs cycle or the synthesis of fat from coenzyme A (Welt and Wilhelmi, 1950; Gurin and Brady, 1953). The metabolic processes regulated by growth hormone obviously couple protein synthesis to the metabolism of fats and carbohydrates. Within this metabolic framework, insulin, the cortical hormones, and thyroxine play a crucial part.

Insulin, for example, cannot participate equally intensively in facilitating the storage of glycogen, fat synthesis, fat storage, oxidation of carbohydrates, and protein synthesis without leading to a metabolic upheaval. Modulation of these several functions of insulin is achieved by the secretory activity of the other endocrine glands. In one endocrine setting, therefore, insulin may promote protein synthesis, while in another it may promote fat synthesis and storage, as we have seen in the case of the cortisone-treated hypophysectomized baboons. Acceleration of metabolism by excess thyroxine not only depletes the fat depots but also speeds up oxidation in the cortisone-treated hypophysectomized or stalk-sectioned baboons, and thus prevents the fatty liver. Both fat and carbohydrates are actively burnt.

Similarly, excess thyroxine arrests the growth of young baboons but induces weight loss in adult baboons (Gillman and Gilbert, 1956d). Then, too, the emphasis on protein synthesis resulting from the administration of growth hormone has repercussions on carbohydrate and on fat metabolism (Greenbaum, 1955; Engel, 1955), and therefore on the precise participation of insulin in metabolism as a whole. Greenbaum and McLean (1953a, 1953b) consider the accelerated fat mobilization as essential for the protein anabolic reaction to growth hormone. The central place of insulin in metabolism is therefore abundantly evident, especially as in the ultimate analysis the energy for all synthetic processes is derived from the oxidation of fats, carbohydrates, and proteins, and in these oxidative processes insulin is indispensable.

In viewing the role of any one hormone in fat metabolism as it affects particularly the development or the prevention of the fatty liver, the overall metabolic context, briefly indicated above, needs to be taken into account. However, it is equally clear that, whichever way a particular hormone alters metabolism, the fatty liver does not emerge in baboons unless insulin is relatively or absolutely insufficient and there is available an adequate amount of cortical hormone. If insulin is absolutely insufficient, as in depancreatized baboons, thyroxine certainly and probably also growth hormone will aggravate the fatty liver.

Fatty Liver of Endocrine Origin and Fatty Liver of Malnourished Human Infants

At the outset it may be stated that the exocrine part of the pancreas does not seem to regulate the fat content of the liver. In depancreatized baboons the amount of available insulin, rather than the pancreatic secretion, was the limiting factor in the production of the fatty liver. The atrophy of the pancreatic acini in malnourished infants may interfere with the absorption of food, and thus indirectly, via the endocrine glands, may affect the evolution of the fatty change.

No significant morphological difference can be found between the intensely fatty liver evoked in baboons by manipulating the endocrine glands and the spontaneously occurring fatty liver of the kind seen in acutely ill malnourished African infants. Indeed, during the progression and regression of the fatty change in baboons, the movement of fat into and out of the cells of the liver lobule follows a pattern similar to that we have observed in human infants. It therefore remains to ascertain whether there is any evidence in favour of the view here presented that the fatty liver of malnourished African infants is aetiologically similar to the kind described above in baboons.

As already mentioned in greater detail elsewhere (Gillman and Gillman, 1951), the fatty liver in African infants evolves rapidly and represents one of the manifestations of a partial metabolic disorganization occurring in an infant with a background of malnutrition. Malnutrition is required to produce a metabolic context in which a mild infection or some other minor stress at least will result in a relative or absolute deficiency of insulin in association with a relative or absolute hyperfunction of the adrenal cortex.

The insulin content of the pancreas of animals may be reduced either by a caloric insufficiency or by the substitution of a poor protein like gelatin for casein in an otherwise adequate diet (Best *et al.*, 1939; Haist *et al.*, 1940; Haist, 1944), or by a deficiency of methionine or cystine (Griffiths, 1950). A sudden loss of appetite, with refusal of food and consequent starvation, such as often happens in malnourished infants early during the acute episode, can reduce the insulin in the pancreas as occurs in animals (Haist, 1944).

The adrenal glands can also be modified by diet, as is clearly evident from the classical studies of McCarrison (1921) on pigeons, guinea-pigs, and monkeys. McCarrison drew attention to the greater sensitivity of the adrenal glands of younger than of older animals to the effects of malnutrition. He stated, moreover, that "the functional perfection of the adrenal gland is dependent on the quality of food." A deficiency of pantothenic acid (Ashburn, 1940; Melampy *et al.*, 1951; Winters *et al.*, 1952a, 1952b; Cowgill *et al.*, 1952), pyridoxine and aneurine (Antopol and Unna, 1942; Deane and Shaw, 1947), and vitamin C (Pfander and Mitchell, 1951) are all said to modify the structural integrity of the adrenal gland to a greater or lesser extent. Apparently, enlargement of the adrenal gland is more marked in partial than in complete inanition (McCarrison, 1921; Jackson, 1925). Hyperactivity of the adrenal cortex culminating in adrenal apoplexy, in association with hyalinization of the islets of Langerhans and dissociated pituitary function, has been described in baboons (Gillman and Gilbert, 1956e). Atrophy of the mucous membrane of the gastro-intestinal canal, the pancreas, and the salivary glands in fatal cases of kwashiorkor and infantile pellagra is reminiscent of similar phenomena in rats after hypophysectomy (Baker and Abrams, 1955). But the not-infrequent hyperactive adrenal cortex, as seen morphologically, recalls the dissociated activity of the pituitary gland described in malnourished baboons. There is more than suggestive evidence that both the islets and the adrenal cortex can be affected profoundly by diet.

During the acute episode in malnourished African infants the ensuing loss of appetite with consequent reduction in caloric intake can still further depress the insulin content of the pancreas, thus satisfying an essential condition for the production of the fatty liver. The slight intolerance to carbohydrate, detected after intravenously administered glucose (Niemeyer and Meneghello, 1950), the apparent inability of the liver to store glycogen (Holmes and Trowell, 1948), and the lipaemia in cases of kwashiorkor terminating fatally (Jayasekera *et al.*, 1951) are all consistent with an absolute or relative insufficiency of insulin. Then, too, the vacuolated nuclei observed in the livers of recovering infants (Gillman and Gillman, 1951) are reminiscent of those

described in depancreatized baboons, and in hypophysectomized baboons chronically treated with cortisone. Such vacuolated nuclei are dependent on a measure of adrenocortical activity and on a relative or absolute insufficiency of insulin (unpublished data).

The information obtained from hypophysectomized or stalk-sectioned baboons developing a fatty liver after withdrawal of cortisone and thyroxine shows that neither the blood fats nor the resting blood sugar need be elevated, although an intolerance to carbohydrate can be demonstrated by the intravenous glucose-tolerance test. It is therefore unnecessary to presume a marked lipaemia or hyperglycaemia in all instances where there is a relative or absolute insufficiency of insulin.

As mentioned above, the atrophy of the lymphoid tissue and thymus, the oedema, and the intolerance of carbohydrate are suggestive of hyperactivity of the adrenal cortex. In terms of the experiments on baboons, even in the presence of normal adrenal function, a depression in insulin secretion would be sufficient to promote a fatty liver.

From the foregoing it is evident that the acutely developing fatty liver of malnourished infants may be immediately related causally to a disturbance of the dynamic relationship between insulin and the adrenocortical and thyroid hormones and not immediately to atrophy of the pancreatic acini. Although the disorder in fat metabolism is manifestly enacted in the liver, the cause for this fatty liver is apparently to be found not in the liver but in the endocrine glands. Finally, this investigation emphasizes the close relation between diet and the physiology of the endocrine glands, a relation which would well repay further investigation if other fatty livers having a seemingly different aetiology from that described in malnourished infants are to be understood.

Summary

Fatty liver has been produced consistently in the baboon by various endocrine techniques. Irrespective of the way a particular hormone altered metabolism, a fatty liver did not emerge unless insulin was relatively or absolutely insufficient and an adequate amount of adrenocortical hormone was available. It was demonstrated that in defined circumstances thyroxine could promote the resolution of a fatty liver.

Amongst their other metabolic effects, insulin and cortisone stimulated fat synthesis and storage in the depots, while insulin and excess thyroxine, even in the presence of excess cortisone, promoted fat mobilization and oxidation. A fatty liver was shown to be dependent at least on a dynamic relationship between insulin, the thyroid, and adrenocortical hormones.

It is suggested that the acutely developing fatty liver of malnourished African infants might be related to an acute endocrine disturbance involving the islets of Langerhans, the adrenal cortex, and the thyroid gland, and not necessarily to atrophy of the exocrine part of the pancreas.

We wish to thank Dr. E. Epstein for carrying out hypophysectomies and Mr. J. C. Allan, of the department of surgery, for performing the pancreatectomies on the baboons.

ADDENDUM.—Since this paper went to press we have been able to show that a saline suspension of acetone dried human anterior pituitary is capable of producing an intensely fatty liver, accompanied by severe constitutional reactions, within five days in a D.-H. baboon not receiving insulin. The contribution of somatotrophic hormone in conjunction with corticotrophin to the fatty liver of human infants still needs to be assessed within the endocrine context discussed above.

REFERENCES

- Antopol, W., and Unna, K. (1942). *Arch. Path. (Chicago)*, 33, 241.
- Ashburn, L. L. (1940). *Publ. Hlth Rep. (Wash.)*, 55, 1337.
- Baker, B. L., and Abrams, G. D. (1955). In *The Hypophyseal Growth Hormone: Nature and Actions*, edited by R. W. Smith, O. H. Gaebler, and C. N. H. Long, Chap. 6, p. 107. McGraw-Hill, New York and London.
- Barrett, H. M., Best, C. H., and Ridout, J. H. (1938). *J. Physiol. (Lond.)*, 93, 367.
- Best, C. H., Haist, R. E., and Ridout, J. H. (1939). *Ibid.*, 97, 107.
- Bornstein, J. (1953). *J. biol. Chem.*, 205, 513.
- Campbell, J. C., Munroe, J. S., Hausler, H. R., and Davidson, I. W. F. (1953). *Endocrinology*, 53, 549.
- (1954). In *Experimental Diabetes*, p. 230. Blackwell, Oxford.
- Chalkoff, I. L., Entenman, C., and Montgomery, M. L. (1947). *J. biol. Chem.*, 168, 177.
- Gillman, T., Entenman, C., Rinehart, J. F., and Reichert, F. L. (1948). *J. exp. Med.*, 88, 1.
- Colowick, S. P., Cori, G. T., and Slein, M. W. (1947). *J. biol. Chem.*, 168, 583.
- Cowgill, G. R., Winters, R. W., Schultz, R. B., and Krehl, W. A. (1952). *Int. Z. Vitaminforsch.*, 23, 275.
- Davies, J. N. P. (1948). *Lancet*, 1, 317.
- Deane, H. W., and Shaw, J. H. (1947). *J. Nutr.*, 34, 1.
- Deuel, H. J. (1954). *Progress in the Chemistry of Fats and Other Lipids*, 2, 140, edited by R. T. Holman, W. O. Lundberg, and T. Malkin. Pergamon Press, London.
- Dragstedt, L. R., van Prohaska, J., and Harms, H. P. (1936). *Amer. J. Physiol.*, 117, 175.
- Engel, F. L. (1955). In *The Hypophyseal Growth Hormone: Nature and Actions*, edited by R. W. Smith, O. H. Gaebler, and C. N. H. Long, Chap. 20, p. 344. McGraw-Hill, New York and London.
- Gilbert, C., and Gillman, J. (1956). *Endocrinology*, 58, 753.
- (1957). *Ibid.*, 60, 570.
- Gillman, J., and Gilbert, C. (1956a). *Nature (Lond.)*, 177, 666.
- (1956b). *Exp. Med. Surg.*, 14, 57.
- (1956c). *Experientia (Basel)*, 12, 65.
- (1956d). *Exp. Med. Surg.*, 14, 48.
- (1956e). *S. Afr. J. med. Sci.*, 21, 46.
- (1956f). *Ibid.*, 21, 89.
- Epstein, E., and Allan, J. C. (1957). *Experientia (Basel)*. In press.
- and Trout, G. E. (1957a). *S. Afr. J. med. Sci.*, 22, 38.
- and Gillman, T. (1945). *Arch. Path. (Chicago)*, 40, 239.
- (1951). *Perspective in Human Malnutrition*. Grune and Stratton, New York.
- Greenbaum, A. L. (1955). In *The Hypophyseal Growth Hormone: Nature and Actions*, edited by R. W. Smith, O. H. Gaebler, and C. N. H. Long, Chap. 19. McGraw-Hill, New York and London.
- and McLean, P. (1953a). *Biochem. J.*, 54, 407.
- (1953b). *Ibid.*, 54, 413.
- Griffiths, M. (1950). *J. biol. Chem.*, 184, 289.
- Gurin, S., and Brady, R. O. (1953). *Recent Progr. Hormone Res.*, 8, 571.
- Haist, R. E. (1944). *Physiol. Rev.*, 24, 409.
- Campbell, J., and Best, C. H. (1940). *New Engl. J. Med.*, 223, 607.
- Holmes, E. G., and Trowell, H. C. (1948). *Lancet*, 1, 395.
- Jackson, C. M. (1925). *The Effects of Inanition and Malnutrition upon Growth and Structure*. Blakiston, Philadelphia.
- Jayasekera, H. T., De Mel, B. V., and Cullumbine, H. (1951). *Ceylon J. med. Sci.*, 8, 1.
- Levin, L., and Farber, R. K. (1951). *Trans. N.Y. Acad. Sci.*, ser. 2, 13, 260.
- Long, C. N. H., and Lukens, F. D. W. (1936). *J. exp. Med.*, 63, 465.
- Louhatieres, A. (1954). In *Experimental Diabetes*, p. 109. Blackwell, Oxford.
- McCarrison, R. (1921). *Studies in Deficiency Disease*. Frowde and Hodder and Stoughton, London.
- Melampy, R. M., Cheng, D. W., and Northrop, L. C. (1951). *Proc. Soc. exp. Biol. (N.Y.)*, 176, 24.
- Niemeyer, H., and Meneghello, J. (1950). *Amer. J. Dis. Child.*, 80, 898.
- Pfander, W. H., and Mitchell, H. H. (1951). *Fed. Proc.*, 10, 391.
- Prohaska, J. van, Dragstedt, L. R., and Harms, H. P. (1936). *Amer. J. Physiol.*, 117, 166.
- Russell, J. A. (1955). In *The Hypophyseal Growth Hormone: Nature and Actions*, edited by R. W. Smith, O. H. Gaebler, and C. N. H. Long, Chap. 2, p. 17. McGraw-Hill, New York and London.
- and Cappelletti, M. (1949). *Endocrinology*, 44, 333.
- Stetten, D., and Salcedo, J. (1944). *J. biol. Chem.*, 156, 27.
- Szego, C. M., and White, A. (1949). *Endocrinology*, 44, 150.
- Trowell, H. C., Davies, J. N. P., and Dean, R. F. A. (1954). *Kwashiorkor*. Arnold, London.
- van Zyl, A. (1955). *S. Afr. J. med. Sci.*, 20, 30.
- (1956). *Physiological Studies on the Protein-bound Iodine and Lipid Metabolism in the Baboon*. Ph.D. thesis, University of the Witwatersrand.
- Weit, L. D., and Wilhelm, A. E. (1950). *Yale J. Biol. Med.*, 23, 99.
- Wertheimer, E., and Shapiro, B. (1948). *Physiol. Rev.*, 28, 451.
- Winters, R. W., Schultz, R. B., and Krehl, W. A. (1952a). *Endocrinology*, 50, 388.
- (1952b). *Ibid.*, 50, 377.
- Young, F. G. (1953). *Recent Progr. Hormone Res.*, 8, 471.

For the past year a new residential school for spastics, "Scotsraig," has been open in Paisley. It is under the direction of the Scottish Council for the Care of Spastics. Once extensions have been completed, the school should have enough places to educate and treat suitable children from the West of Scotland. Cases are screened before acceptance by a panel consisting of an orthopaedic surgeon, a paediatrician, a child psychiatrist, and an educational psychologist. Apply for further details to the social worker, "Scotsraig," Park Road, Paisley.

BACTERIAL ENDOCARDITIS

SURVEY OF PATIENTS TREATED BETWEEN 1945 AND 1956

BY

A. ERIC DORMER, M.D., M.R.C.P.

Senior Medical Registrar, Medical Unit,
St. Bartholomew's Hospital, London

Sulphonamides reduced the mortality of bacterial endocarditis from some 99% to 95% (Friedberg, 1950), but with the introduction of penicillin the cure of most patients at last became a possibility. The manner in which penicillin should be employed was elucidated in this country largely as a result of the Medical Research Council trials which were set up in 1945 (Christie, 1946, 1948, 1949; Cates and Christie, 1951). However, the Registrar-General (1955) showed that in 1949 the early mortality of bacterial endocarditis in the majority of teaching hospitals was 25% (of 124 cases), and for a smaller number of regional board hospitals the mortality was 31% (of 26 cases), while in 1951 these figures were still 19% (of 126 cases) and 40% (of 35 cases) respectively. The yearly mortality due to bacterial endocarditis in England and Wales (Registrar-General, 1955) showed a slight decline from 1938 which became steeper after 1944 when penicillin became available, but this improvement affected females to a greater extent than males. Since 1952 the mortality among males has remained more or less constant, while for females there has been a further slight decrease (Fig. 1).

Because of the persistence of bacterial endocarditis as a cause of mortality, it was decided to survey all the patients who had been treated in this hospital for the disease during the period that antibiotics had been freely available. This survey therefore covers the years 1945 to the end of 1956, and concerns a total of 82 patients (58 male and 24 female); their heart

Fig. 1.—Yearly total deaths in England and Wales due to bacterial endocarditis.

TABLE I.—Heart Lesions—All Patients

Rheumatic heart disease ..	..	..	63
Significant aortic disease ..	..	..	37
Simple mitral stenosis ..	..	..	7
Congenital heart disease ..	..	..	13
Ventricular septal defect ..	..	..	7
V.S.D. and pulmonary stenosis ..	..	..	1
Persistent ductus arteriosus ..	..	..	1
Aortic stenosis ..	..	..	3
Multiple defects ..	..	..	1
Spirochaetal aortic disease ..	..	..	2
Arteriosclerotic heart disease ..	..	..	4

lesions are shown in Table I. In a few cases initial investigation or treatment had been started elsewhere. All patients were traced and survivors followed until 1956 or 1957 with the single exception of one female who went abroad in 1954, four months after cure. Among these 82 patients there were, because of second attacks, a total of 97 separate infections, 22 of which