nical errors. Both patients recovered without disturbances except for a slight paresthesia lasting a fortnight.

The experiments reported fully confirm the research of Brown and Harvey in myotonic goats and localize the origin of myotonic response in the muscle itself.

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Biological Incorporation of a Choline Homologue Into Liver Phospholipids

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Several theories have been proposed to account for the lipotropic action of choline, betaine, methionine, inositol, and other compounds which exert similar effects on the deposition of lipids in the liver. Probably the most attractive hypothesis is one which supposes that incorporation of the active agent into the phospholipid molecule facilitates, in some manner not yet entirely understood, the transport and metabolism of fatty acids.

It is well known that choline and inositol[2] occur naturally in phospholipids. Methionine and betaine apparently exert their effect by donating labile methyl groups for biosynthesis of choline. Ingested choline is incorporated relatively rapidly into liver lecithin (*1*).

The fact that a synthetic analogue of choline, arsenocholine, is lipotropic although it does not possess labile methyl groups, supports the suggestion that the lipotropic action of choline involves reactions which utilize the intact molecule rather than just its labile methyl groups. Welch and Landau (*6*) have shown that dietary arsenocholine is incorporated into the molecule of liver phospholipids, and to them goes the credit for having first stated clearly the arguments in support of the hypothesis outlined above.

More than 10 years ago, however, Channon and Smith (*3*) proposed to test such an hypothesis, viz., that choline exerts its lipotropic action through favoring synthesis of lecithin. Tracer elements being unavailable at that time, a suitable "tracer compound" was sought—an unnatural basic substance with lipotropic properties similar to choline. If such a compound could be found, Channon and Smith suggested trying to discover whether it was incorporated into a new phospholipid molecule in place of the choline of lecithin. They made the triethyl homologue of choline and reported its lipotropic activity. In 1937 Channon, Platt, Loach, and Smith (*2*) attempted unsuccessfully to demonstrate the presence of this base in the phospholipid fraction of the fat extracted from the livers of rats which had ingested about 12 mg. of the compound daily for 20 days. Their statement that they had "established" the absence of the chloroaurate of the ethyl homologue in the least soluble portion of the gold chloride double salt of the choline fraction of the hydrolyzed liver phospholipid, was unfortunate, since it was unjustified in view of the admitted inadequacies of the method used. The inability of Channon, *et al.* to establish, under their experimental conditions, the presence of the ethyl homologue in the liver phospholipid has delayed the general acceptance of the hypothesis that the lipotropic action of choline involves reactions which utilize the intact molecule.

A critical study of their protocols led us to reinvestigate the matter. By feeding larger daily doses of tri-ethyl-β-hydroxyethyl ammonium chloride for a longer period and utilizing new fractionation procedures, we have been able to prove that the ethyl homologue of choline is incorporated into the molecule of a liver phospholipid.

After hydrolysis of the isolated liver phospholipids and removal of the fatty acids, a small portion of the solution was analyzed for choline by the ennea-iodide procedure (*4*) and by the specific microbiological method utilizing the *cholineless* mutant (34486) of *Neurospora crassa* (*5*). A distinct difference in the assay values justified an attempt to prove that the triethyl homologue was present.

The "choline fraction" was precipiated from the hydrolysate with potassium tri-iodide reagent at about 0° C. The bases were freed and oxidized with alkaline permanganate at the boiling point. The resulting tertiary amines were separated by fractional distillation on a microscale and identified as chloroaurates. The finding of trimethylamine (from choline) was anticipated, of course. The isolation of a significant percentage of the fraction as triethylamine, which could have been derived only from the triethyl homologue of choline, proved that this foreign quaternary ammonium base with lipotropic properties had been incorporated into the phospholipids of the liver. Details of this work will be published in the near future.

[1] The writer wishes to acknowledge helpful criticism and suggestions from C. H. Best and C. C. Lucas in the initiation and conduct of this work. The investigation was supported in part by a grant from the Banting Research Foundation.

[2] Although the presence of inositol in liver phospholipids has not yet been reported, a fraction containing inositol has been obtained in this laboratory from a rat liver "cephalin" fraction and is being investigated by L. B. Macpherson and C. C. Lucas.

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The Effect of Alloxan on the *in Vitro* Formation of Glucose by the Liver[1]

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Houssay, Orías, and Sara have recently stated (*3*) that the initial hyperglycemia of alloxan diabetes, which fails to occur in hepatectomized animals, is observed after adrenalectomy or section of the splanchnic nerves, and they expressed the view that the phenomenon must be attributed principally to direct action of alloxan on the liver. Goldner and Gomori (*2*) and Kirschbaum, Wells, and Mollander (*4*) were unable to demonstrate an initial hyperglycemia in dogs and rats, respectively, after adrenalectomy.

The experiments here reported may throw some light on the direct effect of alloxan on the liver. Respiring rat liver slices were suspended in 3 cc. of a glucose-free modified phosphate-buffered Ringer's solution (MPBR) to which, in certain of the flasks, alloxan was added in 0.0014 M concentration, which is roughly equivalent to the concentration that would occur if there is an equal distribution throughout the body after a subcutaneous injection of 200 mg./kilo. The amount of glucose (as total reducing substances by the ferricyanide method of Folin) appearing in the medium after one hour's shaking in oxygen at 37° C. was measured, with the results given in the table.

Each figure represents the average of three observations. Control and alloxan-treated tissues of each experiment were taken from the pooled liver slices of the same animal. The glucose appearing in the medium was the same, within the limits of error, in the alloxan-treated tissues as in the controls. Assuming that the dry weight of rat liver is 28 to 30 per cent of the wet weight, it can be calculated that the glucose in the medium represented about 6 per cent by weight of carbohydrate in the liver slices. Only glycogen could have been the source of most of this material.

In four animals made diabetic by a subcutaneous injection of 200 mg. alloxan/kilo, 48 hours previously, the amount of glucose appearing in the medium is less than one-fifth of the amount seen in the case of the normal liver. Lackey, Bunde, Gill, and Harris (*5*) have shown that in alloxanized rats with moderate to severe diabetes the liver glycogen is low, and we attribute the lessened formation of glucose in the diabetic livers to this fact.

Exp. No.	Blood sugar in mg. %	Liver slices in MPBR: Dry wt. of tissue (mg.)	Liver slices in MPBR: Glucose in medium (mg.)	Liver slices in MPBR: Glucose/mg. dry wt. of tissue (μg.)	Liver slices in MPBR plus alloxan: Dry wt. of tissue (mg.)	Liver slices in MPBR plus alloxan: Glucose in medium (mg.)	Liver slices in MPBR plus alloxan: Glucose/mg. dry wt. of tissue (μg.)
Normal Rats							
1		15.0	4.35	290	16.2	4.20	259
2		17.2	2.55	148	14.0	2.86	204
3		12.9	2.55	197	13.6	2.62	193
4		14.9	2.70	181	15.5	2.92	188
5		16.6	2.85	172	16.1	2.52	156
6		11.6	2.32	200	11.7	2.85	243
		Average		198 ± 20	Average		207 ± 15
Diabetic (Alloxan) Rats							
7	520	13.8	0.345	25	12.9	0.368	28
8	520	13.0	0.555	43	11.8	0.435	37
9	760	16.1	0.270	17	15.7	0.330	21
10	450	12.5	0.600	48	13.2	0.720	55
		Average		33 ± 7	Average		37 ± 8

Our results indicate that alloxan has no direct effect on liver glycogenolysis and are therefore opposed to the concept of Houssay, *et al.* in regard to the origin of the initial hyperglycemia of alloxan diabetes. The possibility remains that alloxan may act upon the liver via nervous pathways, as suggested by the recent observations of Braulio (*1*) in the cat.

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[1] Aided by grants from the Committee on Research in Endocrinology of the National Research Council and the Charlton Fund.