The experiments on the heart-lung preparation of the dog clearly demonstrated the marked antidotal properties of sulfhydryl-containing compounds against the negative inotropic action of Salyrgan. In Table 1 it can be seen that severe heart failure produced by 150 mg. of Salyrgan could be promptly counteracted by 100 mg. of glutathione. Similar effects were

TABLE 1

THE EFFECT OF GLUTATHIONE ON HEART FAILURE PRODUCED BY TOXIC DOSES OF SALYRGAN*

Time in minutes	Systemic output cc./min.	Aortic pressure mm./Hg	Pulmonary pressure mm./H$_2$O	Right auricular pressure mm./H$_2$O	Left auricular pressure mm./H$_2$O	Heart rate per min.
0	400	105	136	19	32	140
5	Salyrgan, 50 mg.					
7	400	105	136	19	32	140
9	Salyrgan, 50 mg.					
11	400	105	136	19	32	140
14	Salyrgan, 50 mg.					
15	370	105	155	25	108	140
16	320	102	250	45	320	
18	100	95	545	94	478	
18.5	50	95	623	100	523	150
19	Glutathione, 100 mg.					
19.5	250	95	409	65	377	156
21	500	110	136	19	32	140
22	400	105	136	19	32	140
24	400	105	136	19	32	140
49	400	105	140	19	35	140

* Heart-lung preparation. Female dog, 10.6 kg. Arterial resistance, 72 mm. of mercury. Blood temperature, 39.0° C. Blood volume, 880 cc. Experiment 2, 14 January 1946.

obtained with 56 mg. of cysteine hydrochloride and 10 mg. of 2,3-dimercapto-propanol. Cystine in doses up to 200–300 mg. did not counteract Salyrgan-induced heart failure. Furthermore, in spontaneous heart failure and heart failure induced by sodium pentobarbital, neither cysteine, glutathione, nor 2,3-dimercapto-propanol had any positive inotropic effect.

SUMMARY

The acute toxicity of an organic mercurial as studied on the mouse, intact anesthetized cat and dog, and the heart-lung preparation of the dog can be readily counteracted by sulfhydryl-containing compounds such as glutathione, cysteine, and 2,3-dimercapto-propanol. Equimolar doses of 2,3-dimercapto-propanol are about five times as active as cysteine or glutathione.

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The Mechanism of Myotonic Contraction

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In 1942, while reviewing the mechanism of myotonias (*5*), the writer concluded that there were several reasons for admitting that the delayed relaxation of myotonic muscle was due to a pathological state of the muscle itself, independent of the central nervous system and of the neuromuscular synapsis.

The most important demonstration of the exclusion of the central nervous system has been furnished by Grund (*3*), Schäffer (*6*), Kennedy and Wolf (*4*), and Denny-Brown and Nevin (*2*), who showed that after nerve or spinal anesthesia, the myotonic reaction still exists in the anesthetized limb. Referring to the synapsis, Brown and Harvey (*1*) pointed out that in myotonic goats the completely curarized muscle shows myotonic phenomena.

The last experiment is crucial, and the only criticism of its generalization is that the experiments were performed on a condition supposed to be similar to that of myotonia in human beings. For these reasons, and counting on the willing cooperation of two patients affected by *Dystrophia myotonica*, experiments were planned to discover the effect of the exclusion of the synaptic mechanism in these patients.

The experiments were performed under penthotal anesthesia. Two shielded electrodes were placed in the median nerve, and two needle electrodes punctured the proximal and distal part of the thenar eminence, respectively. The nerve was stimulated supramaximally by a thyratron stimulator, and the source of the needle electrodes was an a-c potential reductor. The registration was recorded in a smoke drum through a myograph attached to the interphalangic joint of the first finger.

The experiment began with a two-second direct and indirect stimulation of 60 stimuli per second. A suitable dose of *Curare Brasiliensis* injected in the brachial artery, with a cuff to avoid the venous circulation during two minutes after the injection, completely suppressed the effect of the nerve stimulation. The direct stimulation, conversely, reproduced exactly the previous registration, showing that the myotonic delayed relaxation was not influenced by the synaptic exclusion. Also, the percussion of the muscles of the thenar eminence showed the same myotonic responses after full curarization.

In the second patient the effect of variations of frequency of stimulation was studied in addition, but a total curarization was not obtained because of tech-

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.

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[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.