sulfate (NICHOLAS et al.[2]). This material, precipitated three times between 40 and 60% saturated ammonium sulfate, is used after dialysis against *M*/15 potassium phosphate buffer at pH 7 and sterilization by filtration through asbestos pads (Hercules ST grade).

The effectiveness of this supplement has now been found to be increased about 50 fold upon freezing it at 2% by volume with the basal medium. Activation does not occur at 4°C, 20°C, or by preincubation at 37°C, but only upon freezing. Freezing conditions of −17°C for periods of one day to two weeks have been used with no apparent differences in effectiveness. The activation is lost by holding at 4°C overnight, but is restored by refreezing; the process, then, is a reversible one. Preliminary experiments indicate that a shift in a peak of the absorption spectrum accompanies activation, as well as a change in optical rotation.

As shown in a previous paper (DOUGHERTY and HANSEN[3]), the concentration of a growth requirement can be related by the usual log dose-response curve to the number of days a newly hatched larva takes to reach maturity. Young larvae hatched into phosphate buffer were inoculated into medium immediately after it had thawed and warmed to 20°C, and the rate of growth to maturity determined (DOUGHERTY et al.[4]). The activated supplement permitted successful growth and reproduction when added to our chemically defined basal medium in an amount corresponding to 5 μg of nitrogen per culture.

Six preparations of this protein fraction, two from horse liver and four from lamb liver, have shown this 'freeze-activation'. These ranged in dry weight 34–73 mg/ml, nitrogen content 3.6–6.8 mg/ml, optical density ratio at 280/260 mμ– 1.31–1.57.

Preliminary attempts to activate supplements from chick embryo extract, serum, and autolyzed bacteria have been unsuccessful so far.

The 'freeze-activation' of the liver protein fraction did not occur in water, but initially, only with the chemically defined medium. This is a multi-component medium, designed for maximum growth response of the nematode (in publication). Since it seemed likely that only a part of the medium was involved in the activation, the components were investigated first in groups and then singly. Freezing with amino acids, salts, vitamins, or glutathione was ineffective. Only the nucleotide-group produced activation, and it was subsequently obtained with each of the nucleotides (adenylic, guanylic, cytidylic, and uridylic acids) and the base (thymine), tested individually at 1 *mM* concentration, their level in the basal medium (Table). The final tests were made by freezing these solutions with 2% liver protein fraction and then adding the activated mixture to an equal volume of chemically defined medium at 2 fold concentration.

The nature of this 'freeze-activation' process remains obscure; there is clearly an interaction between some component of the supplement and one of the nucleotides or the nucleic acid base during freezing and thawing. This interaction could be: 1. an alteration of some spatial configuration by rearrangement of hydrogen bonding to make the material more accessible to the enzyme systems of the nematode, 2. the displacement of some small molecule from a more complex material, or 3. an unfolding of the protein and subsequent interaction with the heterocyclic activator to produce a new and transient material of high biological activity.

An interesting speculation is that this 'freeze-activation' could, perhaps, play a significant role in the survival of micrometazoa in the polar regions.

Activation of liver protein fraction as a nutritional supplement by freezing and thawing

Liver protein[a] %	Activator	Days to mature	
		Freeze-activated	Fresh Mix
10	EM-62[b]	21	21
5	EM-62	7	non-maturing
2	EM-62	5	non-maturing
1	EM-62	5	
0.5	EM-62	11	
0.25	EM-62	non-maturing	
0.1	EM-62	non-maturing	
1	4 nucleotides[c] + thymine	7	
1	individual nucleotides[c] or thymine	8–9	
1	EM (less nucleotides and thymine)	non-maturing	

Concentrations of supplement less than 2% were equally effective whether obtained by diluting the 'freeze-activated' 2% mixture or by freezing at lower dilutions.

[a] Fraction-C; dry weight 38.3 mg/ml; N 4.6 mg/ml; O.D. 280/260 mμ = 1.53.

[b] Chemically defined medium containing amino acids, vitamins, salts, nucleotides, glucose, and glutathione (in publication).

[c] Adenylic, guanylic, cytidylic, and uridylic acids.

Résumé. Une protéine du foie, en partie purifiée, congelée en présence de nucléotides ou de thymine a montré un accroissement d'activité. Ceci résulte en une augmentation de 50 fois la valeur nutritive, lorsque ajoutée au milieu chimique défini pour la culture du nématode *Caenorhabiditis briggsae*. Un changement physique de la protéine est postulé, à cause du changement de la rotation optique de l'absorption aux UV qui accompagne l'accroissement d'activité.

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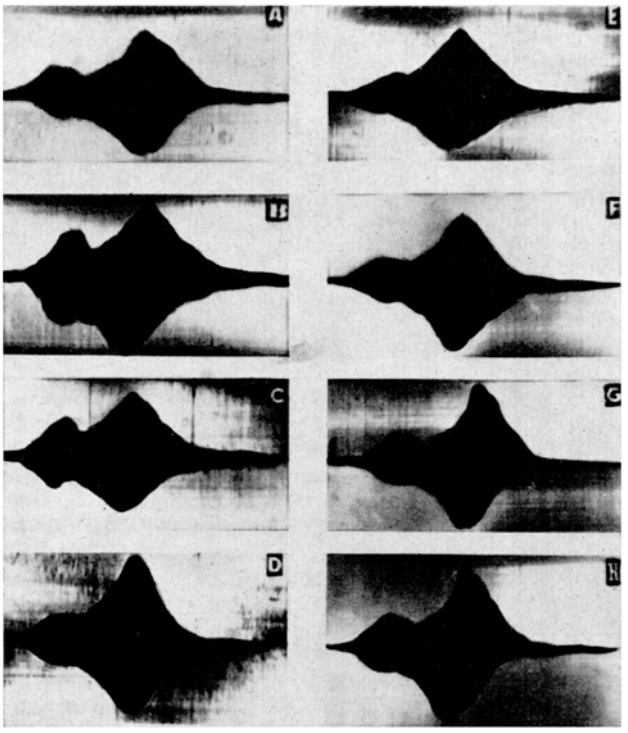
Changes in the Liver Protein Pattern due to Lysergic Acid Diethylamide (LSD)

While studying the effects of lysergic acid diethylamide (LSD), we carried out some investigations on the electrophoretic pattern of organ-extracted proteins. Although such studies are still in course, we should like to anticipate a few data concerning the behaviour of proteins extractable from liver by low ionic strength buffers. In fact, 1 mg/kg of the drug injected intraperitoneally causes a sudden and remarkable change of the free electrophoretic protein pattern so that any attempt to calculate the mobility values is often unreliable (Fig.).



Free electrophoresis of rat liver proteins extracted after intraperitoneal injection of 1 mg/kg LSD. Extraction in phosphate Na buffer, pH 7.2, i.s. 0.1. Migration through 10 100 sec in Na-veronal/Na acetate, pH 8.6, i.s. 0.1. The following protein patterns are from animals killed 0, 10, 30, 45, 90, 180, 1080, and 1440 min respectively after the pharmacological treatment.

The phenomenon, completely reversible within 24 h, is most probably connected with what KEUP[1] said on the incorporation of labelled LSD into liver proteins. Another correlation might also be supposed: the one between hepatic lesion and central phenomena induced by LSD, according to the schema previously described by BLOCH et al.[2] as regards the mechanism of action of trimethyloxyphenylethylamine.

What we should like to stress particularly are the following points:

1. The effect of LSD on the liver would not appear to be strictly specific, as it is reproduced also by 2-bromodiethylamide LSD.

2. The change appears whenever the drug is administered and, though the repetition of doses makes it decrease, such decrease never reaches the degree observable for central responses.

3. The chronological development of the biological phenomenon (Fig.) corresponds to the results obtained concerning the drug distribution in the metabolism[3–5] and to those of KEUP on the incorporation of labelled LSD into the liver proteins; all such data show that the liver concentration starts almost immediately after intraperitoneal or intravenous injection and reaches its maximum within 2–4 h.

Riassunto. Mediante elettroforesi in fase libera è stato studiato il protidogramma epatico durante l'intossicazione con dietilamide dell'acido lisergico (LSD) nel ratto albino.

Si sono osservate profonde modificazioni del quadro le quali sono completamente reversibili.

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Beitrag zur *in vitro*-Wirkung eines Thiapyronderivates auf die Tributyrin und Acetylcholin spaltenden Fermente des Rattenserums

In einer Reihe von Arbeiten über die Aktivität der Cholinesterasen sind mehrere Hemmstoffe identifiziert worden, die besonderes pharmakologisches Interesse beanspruchen. Zu ihnen zählen beispielsweise Eserin und Physostigmin[1–3], Tri-*o*-kresylphosphat und Morphin[4], einige Cucarepräparate[5], Diisopropylfluorophosphat[6], Parathion u. a.

In Stoffwechselversuchen, die wir in unserem Institut mit einigen Thiapyronderivaten durchführten[7], konnte die Feststellung gemacht werden, dass diese Verbindungen interessante aktivierende Eigenschaften besitzen. Diese Tatsache veranlasste uns, den Einfluss eines in dieser Beziehung besonders aktiven Thiapyronderivates auf die Spaltfähigkeit von Rattenserum für Acetylcholin und Tributyrin zu untersuchen.

Methodik. Die Versuche wurden an (♀) Wistar-Ratten in einer Gewichtsklasse von 150–200 g durchgeführt. Die Tiere wurden mit einem im VEB «Küssnerwerk» hergestellten Nährkonzentrat ernährt. Als Testsubstanz benutzten wir das 3-Benzyl-4-hydroxy-5-carbäthoxy-6-methylthiapyron[1,8], die wir als A_1 bezeichneten. Die Bestimmung der Fermentaktivität erfolgte auf manometrischem Wege. In allen Fällen dienten Leeransätze zur Ermittlung der Spontanhydrolyse des Substrates bzw. der ohne Gegenwart von Substrat aus dem Puffer in Freiheit gesetzten CO_2-Menge. Die Reaktion verläuft bei geeigneter Temperatur von 38° C und in Ringerlösung R_{30}. Das Substrat wurde als 2%ige Tributyrinlösung bzw. 1%ige Acetylcholinlösung (0,2 ml Tributyrin ad 10 ml R_{30}, 100 mg Acetylcholin ad 10 ml R_{30}) in einer Menge von 1,5 ml/Ansatz eingesetzt. Als Enzym verwendeten wir 0,5 ml einer 15%igen Serumverdünnung, die in den Seitenarm des Warburg-Gefässes einpipettiert wurde. Die Prüfsubstanz wurde in R_{30} zugegeben.

Diskussion. Aus den tabellarisch dargestellten Befunden ist ersichtlich, dass das untersuchte Thiapyronderivat die fermentative Hydrolyse von Tributyrin zu hemmen vermag. Da eine relativ grosse Anzahl von Autoren bislang

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