It was capable of hydrolysing starch and decomposing gelatin and casein.

Cultures for antibiotic production were made in a medium containing (per cent w/v) 0·25 peptone, 0·25 meat extract, 1·0 glucose, 0·5 sodium chloride and 1 per cent (v/v) corn steep liquor (40 per cent solids), in a 20-litre tank at 27° C. These were grown for 72 hr., constantly stirred, and aerated with 15–20 litres/min. of air.

The medium was then filtered and extracted with ethyl acetate, the solvent dried, reduced *in vacuo* and washed in dilute sodium hydroxide. The ethyl acetate was further reduced in volume and passed through a short column of an intimate mixture of 'Darco *G* 60' and 'Hyflo-Supercel' (1 : 1). After elution with ethyl acetate and concentration, the crude complex was precipitated by petroleum ether, dissolved in hot carbon tetrachloride and passed through a similar, longer column, eluted with benzene and again precipitated by petroleum ether.

The purified complex was a white, amorphous powder, soluble in lower alcohols, acetone, chloroform and ether, insoluble in water. The percentage composition was approximately: C, 59·5; H, 8·0; N, 2·0 ($c = 1$ per cent in methanol) $[\alpha]_D^{20} = -34·5°$. In the hydrolysate, seven amino-acids were detected: glycine, proline, alanine, valine, phenylalanine, leucine and aminobutyric acid (probably the γ-isomer). The ultra-violet adsorption spectrum showed a maximum at 282 mμ.

By paper chromatography, eight components of the complex could be recognized.

The *LD*50 in albino mice was 400 mgm./kgm. intravenously, 600 mgm./kgm. intraperitoneally and 2 mgm./kgm. subcutaneously.

The complex was active against *Bacillus* spp., staphylococci and hæmolytic streptococci at 0·25 μgm./ml. or less, but was inactive against Gram-negative bacteria and non-hæmolytic streptococci.

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PHYSIOLOGY

Effect of Hydration on Hexobarbitone-induced Sleep in Mice

Hydration of laboratory animals and man produces a complex picture generally indicating increased excitability of the central nervous system. This is attributed to osmotic disturbances produced by fall of the extracellular electrolytes[1]. It is therefore interesting to find in albino mice that after hydration the sensitivity of the central nervous system to hexobarbitone was increased simultaneously. This conclusion is based on the results obtained from the following experiments.

Adult albino mice received intraperitoneally hexobarbitone sodium 100 mgm./kgm. after water-load. Control mice received only hexobarbitone. In some control experiments mice received water (or normal saline or 5 per cent glucose) just after recovering from sleep (Table 1).

About 2–4 hr. before becoming absorbed, intraperitoneally administered 5 per cent glucose solution is known to remove electrolytes from the extracellular body fluid, thus producing a condition comparable to hydration[2]. Mice receiving this treatment were used ½ and 2 hr. later for hexobarbitone sleep. The effect of normal saline was also studied (Table 2).

Table 1. Effect of Hydration on Hexobarbitone Sleeping Time in Mice. Water at 37° C. injected Intraperitoneally. Animals were kept at 32–35° C.

Hydration mil. % of body-weight	Min. between hydration and hexobarb.	Sleeping time min. ± *S.D.*	No. of mice used	Value of *P* (*t* test)
Nil	Nil	21 ± 4·1	20	
5	30	18 ± 6·8	21	*
10	0	36 ± 9·5	12	< 0·01
10	15	56 ± 12·7	15	< 0·01
10	30	49 ± 11·2	20	< 0·01
10	60	40 ± 12·3	15	< 0·01
10	240	26 ± 6·8	8	< 0·05
10	Just after hexobarb. sleep	22 ± 5·5	20	

* Not significant.

Table 2. Effect of 0·9 per cent Sodium Chloride Solution or 5 per cent Glucose at 37° C., injected intraperitoneally, on Hexobarbitone Sleeping Time in Mice. Room Temperature 32 – 35° C.

Fluid mil. % of body-weight	Min. between fluid and hexobarb.	Sleeping time min. ± *S.D.*	No. of mice used	Value of *P* (*t* test)
10 (Glucose)	30	25 ± 5	18	< 0·02
10 (Glucose)	120	54 ± 12·9	18	< 0·01
10 (Saline)	30	22 ± 8·8	18	*
10 (Saline)	120	17 ± 8·4	12	*

* Not significant.

Results indicate that 10 per cent but not 5 per cent hydration prolongs hexobarbitone sleeping time, the maximal effect occurring 15 min. after hydration. The effect was also seen 30 and 60 min. after hydration. The rate of water excretion can explain these results. Animals recovering from sleep go back again to sleep after hydration but not after saline load. This suggests sensitization of the central nervous system and excludes other mechanisms of action. Hydrated mice were quieter but essentially normal in their behaviour. All experiments could be repeated on the same animals after 2–3 days.

Glucose solution, requiring a certain time-interval to deplete the extracellular electrolytes, produces a distinct effect 2 hr. later. Further work is in progress.

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[1] *Lancet*, i, 425 (1953).
[2] Swinyard, E. A., Toman, J. E. P. and Goodman, L. S., *J. Neurophysiol.*, 9, 47 (1946).

Evidence for the Identity of Natriferin, the Frog Water-Balance Principle and Arginine Vasotocin

Maetz, Morel, and Lahlouh[1] recently reported that pituitary extracts from amphibians and teleosts contain a principle which promotes the active transport of sodium by the isolated skin of the frog *Rana esculenta*. This factor cannot be oxytocin or a vasopressin since it has approximately ten times more activity on sodium transport than do mammalian extracts of similar oxytocic activity. Maetz *et al.* named this principle 'natriferin'.

Heller[2,3] first pointed out that fish, amphibian, avian, and reptilian pituitary extracts exert far more effect on frog water-balance than can be attributed to their content of oxytocin or vasopressin. Pickering and