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Effect of Spermine on Tissue Oxidations.*

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(Introduced by F. C. Koch.)

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Little is known of the biological rôle of spermine (N, N'-dipropylamino-diaminobutane) despite its widespread occurrence in mammalian tissues (human semen, bovine pancreas, spleen, thyroid, lung, ovary, brain, ocular tissue, muscle, liver, and intestine). We have isolated spermine from dilute acid extracts of swine duodenal mucosa. The fact that these extracts produce a hyperglycemia in rabbits led us to investigate the effects of the pure base on the blood sugar level. The intramuscular injection of 15-25 mg per kilo of spermine into fasted, male rabbits weighing about 2 kilos, induces a definite and prolonged hyperglycemia.

In order to illuminate further the effects of spermine on the intact organism we have undertaken a general study of the action of the base on the respiration of isolated mammalian tissues. The addition of spermine to guinea-pig brain brei suspended in a phosphate-saline medium has little immediate effect on the respiration as measured by the direct Warburg technic. However, in an extensive series of experiments, a slight increase of oxygen consumption in the presence of spermine was noted, especially during the third and fourth hour following the addition of the amine.

If glucose is present as a substrate for either brain brei or brain slices (cortex), the addition of spermine causes a marked inhibition of the oxygen consumption (for typical experiments see Table I, Exps. 11, 13, 81). This inhibition (calculated as % inhibition of the extra oxygen consumption due to glucose) shows a tendency to decrease with time, especially when brain slices are used. The averaged data from a series of 8 experiments on brain brei gave values of 75, 74, 73, and 65% inhibition of oxygen consumption by 0.0060 M spermine in each of the 4 thirty-minute periods following addition of the amine. With 0.0015 M spermine, the average inhibition for the same periods was 62, 57, 54, and 51% respectively. Spermine also inhibits the respiration of brain brei in the presence of blood serum as a substrate (Table I, Exp. 53). Similar inhibi-

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tion is observed with lactate and pyruvate as substrates (Table I, Exps. 27, 29, 37, 40). At the concentrations used the amine has relatively small effect on the extra oxygen consumption due to the addition of glutamic acid (Table I, Exps. 25, 26). With succinic acid no appreciable effect is demonstrable (Table I, Exp. 15).

The addition of small amounts of spermine to guinea-pig skeletal muscle *in vitro* likewise causes respiratory inhibition although this occurs in the later periods of the experiment. Table II shows the effect of varying concentrations of the amine on the respiration of minced muscle suspended in phosphate buffer containing citric acid and boiled muscle extract. The inhibition can be demonstrated in the absence of both the citrate and the muscle extract, but the total respiration of the tissue is then much smaller. Preliminary experiments indicate that a similar inhibition occurs with strained liver suspensions.

The effect of spermine on glucose, lactate, and pyruvate oxidations in brain superficially resembles the action of various narcotics[1] although spermine does not produce narcosis in the intact animal. Since the oxidative inhibition of the narcotics has been associated with an inhibition of the tissue dehydrogenases, we investigated the possible effect of spermine on these enzymes. Determination of the rate of decoloration of methylene blue (Thunberg technic) by buffered brain brei indicates that spermine causes a small but definite acceleration (ca 20%) in the rate of decoloration, both in the presence and absence of glucose.

The *in vitro* action of spermine on brain oxidations also resembles that of certain amines, notably tyramine, β-phenyl-ethylamine, β-phenyl-β-hydroxy-ethylamine, and mescaline.[2] Neither these substances nor the narcotics are regarded as normal tissue constituents. The facts here presented are of special interest, therefore, in that they demonstrate that spermine, a normal constituent of brain as well as other tissues, has *in vitro* properties similar to those of various pharmacological agents acting on the central nervous system.

Since the systems inhibited by spermine seem to be those which require the presence of co-enzymes, it is possible that spermine may act by interfering with co-enzyme action. This possibility is being investigated.

Associated with spermine in the brain, pancreas, and iris, is a chemically related base, spermidine (N-propylamino-diaminobu-

[1] Quastel, J. H., and Wheatly, A. H. M., *Proc. Roy. Soc.*, B, 1932, **112**, 60.

[2] Quastel, J. H., and Wheatly, A. H. M., *Biochem. J.*, 1933, **27**, 1609.

TABLE I.

Tissue	Substrate	Exp. No.	Molar Conc. Spermine	Time, min.	mm³ O_2 consumed per 100 mg wet wt: Brain	Brain + Spermine	Brain + Substrate	Brain + Spermine + Substrate	% Inhibition	Exp. No.	Molar Conc. Spermine	mm³ O_2 consumed per 100 mg wet wt: Brain	Brain + Spermine	Brain + Substrate	Brain + Spermine + Substrate	% Inhibition
Minced Guinea Pig Whole Brain	.0015 M Glucose	11	.0015	0-60	86.0	87.0	116.0	100.0	57	13	.006	96.0	94.0	120.0	100.0	75
				60-90	34.0	36.0	54.1	44.9	56			33.9	35.0	47.1	38.9	70
				90-120	24.0	27.0	39.9	37.1	36			25.3	32.2	42.9	39.1	61
	.013 M Lactate	29	.0015	0-60	72.0	71.4	120.1	102.5	35	27	.006	72.8	70.9	118.6	93.1	52
				60-90	24.9	26.8	47.8	40.3	41			24.1	28.5	48.8	37.7	63
				90-120	21.9	22.0	41.7	33.4	42			22.3	24.6	40.8	31.5	63
	.013 M Pyruvate	37	.0015	0-60	69.1	74.1	130.2	110.8	40	40	.006	66.7	68.4	126.2	92.8	59
				60-90	24.0	25.6	50.3	38.2	52			26.0	24.6	50.8	31.8	71
				90-120	21.7	21.0	42.4	33.0	42			17.3	24.0	42.8	31.8	69
	.053 M Glutamate	25	.0015	0-60	76.4	73.7	102.1	98.9	2	26	.006	68.4	68.5	102.0	96.3	17
				60-90	22.8	25.5	42.0	40.0	24			23.6	26.3	44.1	40.6	30
				90-120	19.3	21.1	34.3	36.1	0			24.5	28.1	40.0	37.4	40
	.021 M Succinate	15	.0030	0-60	66.5	65.5	92.9	91.7	1							
				60-90	27.0	26.1	38.1	38.5	—12							
				90-120	20.4	22.6	32.2	33.3	9							
	10% guinea pig blood serum	53	.0060	0-60	65.3	73.2	126.6	95.1	64							
				60-90	16.7	22.1	34.5	26.3	76							
				90-120	13.3	24.9	38.1	30.5	77							
Guinea Pig Brain slices (Cortex)	.0015 M Glucose	81*	.0015	0-60	6.38	7.04	16.02	11.59	53							
				60-90	1.33	1.84	6.49	5.86	41							
				90-120	1.15	1.60	4.81	4.69	16							

Contents of vessels: 0.7 cc M/5 phosphate buffer, pH 7.4 + 1.1 cc 0.9% NaCl + 100 mg wet tissue. Other constituents as indicated, all added as neutral salts. 0.1 cc 20% KOH and starch-free filter paper in central cups.

* 0.7 cc M/15 phosphate buffer used. O_2 consumption as mm³ O_2 per mg dry weight of tissue.

tane). Our preliminary studies indicate that this substance possesses properties similar to those reported here for spermine.

TABLE II.

Time, min.	mm³ O_2 consumed* Muscle	Muscle + .0037 M Spermine	Muscle + .0019 M Spermine	Muscle + .00027 M Spermine
0-60	540	517	528	497
60-120	378	210	338	358
120-180	165	33	84	139
180-240	32	21	23	27
Total O_2 consumption	1115	781	973	1021
Inhibition		30%	13%	8%

* Each vessel contains 1.6 cc muscle suspension (cooled guinea pig leg muscle minced and suspended in 5 vl. M/10 phosphate buffer, pH 6.8) + 1.0 cc muscle extract (guinea pig leg muscle minced + 1 volume water, kept in boiling water bath 10 minutes and filtered) + .3 cc 0.2 M sodium citrate + 0.2 cc spermine hydrochloride in 0.9 % NaCl made up to final molarities.

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Studies on the Colloidal Gold Curve of Blood Serum in Liver Disease.

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The quantitative alterations of the plasma proteins in liver disease have been recognized for many years. The work of Gros[1], Kendall[2] and de Vries[3] suggests, however, that a qualitative change in the plasma globulin, as indicated by an increase in the euglobulin fraction, may occur frequently in hepatic disease. These investigations indicate that an increase in euglobulin may distinguish the globulin of liver disease from that of other diseases. Since the plasma globulin[4] and, particularly, the euglobulin[5] have been shown to play an important rôle in colloidal gold precipitation, these studies of the colloidal gold reaction of blood serum in liver disease were undertaken.

1 Gros, W., *Deutsch. Arch. f. klin. Med.*, 1935, **177**, 461.

2 Kendall, F. E., *J. Clin. Investigation*, 1937, **16**, 921.

3 de Vries, A., *Act. med. Scandinav.*, 1938, **98**, 95.

4 Cruickshank, J., *Brit. J. Exp. Path.*, 1920, **1**, 71.

5 Mellanby, J., and Anwyl Davies, T., *Brit. J. Exp. Path.*, 1923, **4**, 132.