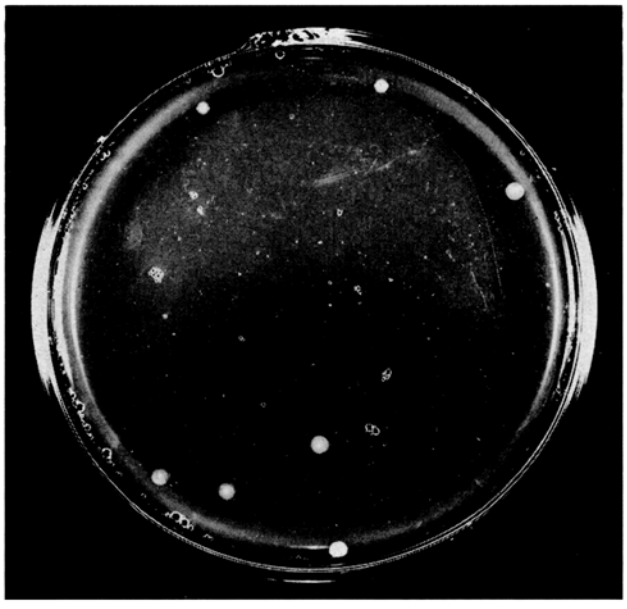

Fig. 3. Arg+ reversions of G-12 induced by UV on minimal A plate with limited broth enrichment.

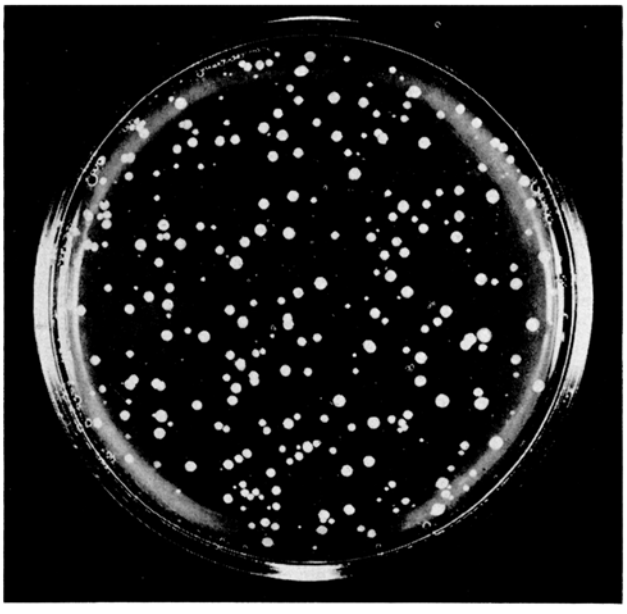

Fig. 4. Reversions of G-12 produced by direct treatment with 0.03 ml of ethyl methane sulphonate on limitedly enriched minimal A medium.

richia coli K-12 strains HfrC and HfrH, with cysteine auxotrophs of *Klebsiella pneumoniae* strains 2 PARK and 8172, and a methionine auxotroph of *K. pneumoniae* strain 418. Strong syntrophism occurred between Salmonella prototrophs and auxotrophs, including G-12, and met⁻ or cys⁻ mutants of Escherichia and Klebsiella, probably due to H_2S production by the Salmonellae[12].

Résumé. L'auteur décrit un mutant auxotrophe pour l'arginine chez *Salmonella typhimurium*. Les prototrophes arg+ sont induits par les rayons UV, $MnCl_2$ et méthane sulfonate d'éthyle. Le β-propiolactone est moins efficace pour l'induction des arg+. La transduction avec le bactériophage PLT-22 cultivé sur une souche prototrophe donne arg+ à une fréquence de $1/10^7$ bactériophages. Le mutant arg⁻ est fécond ni avec les souches d'*Escherichia coli* K-12 HfrC et HfrH ni avec trois souches de *Klebsiella pneumoniae*.

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[12] H. H. Plough, H. Y. Miller, and M. E. Berry, Proc. Nat. Acad. Sci. U.S. *37*, 640 (1951).

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Uptake of C^{14}-Ethionine by Mouse Foetuses[1]

In connection with our investigations on the incorporation of C^{14}-ethionine into the pancreas, surprisingly high accumulation of radioactivity was observed in the foetuses of pregnant mice.

Various investigators have reported that amino acid analogues have specific effects on the growth of the foetus and are toxic for it[2-4]. It has been suggested that this effect is primarily concerned with protein synthesis of the embryonic cell.

The administration of ethionine—the ethyl analogue of methionine—to male or female rats causes acute pancreatic acinar necrosis[5,6]. The pathogenesis of the pancreatic lesions is apparently related to interference in some phase of methionine metabolism since the changes are prevented by the simultaneous administration of methionine.

Ethionine has been given to pregnant rats in an attempt to induce congenital cystic fibrosis of the young pancreas[4]. This attempt failed but many of the young were born dead, had various congenital malformations, or were undersized. Although the maternal pancreases were severely damaged by the ethionine injections, the foetal pancreas remained essentially normal and the authors therefore questioned the passage of the ethionine across the placenta. Against this background, we should like to report our observations on the uptake and nature of the radioactivity observed in the foetuses after administration of C^{14}-ethionine to pregnant mice.

Two days before expected parturition, pregnant albino mice (body weight 30 g) were injected intravenously with 1 μC C^{14}-*DL*-ethionine (Research Specialties, Richmond, Calif., U.S.A., Spec. act. 1.2 mc/m*M*).

[1] This work was supported by grants from the Swedish Medical Research Council.

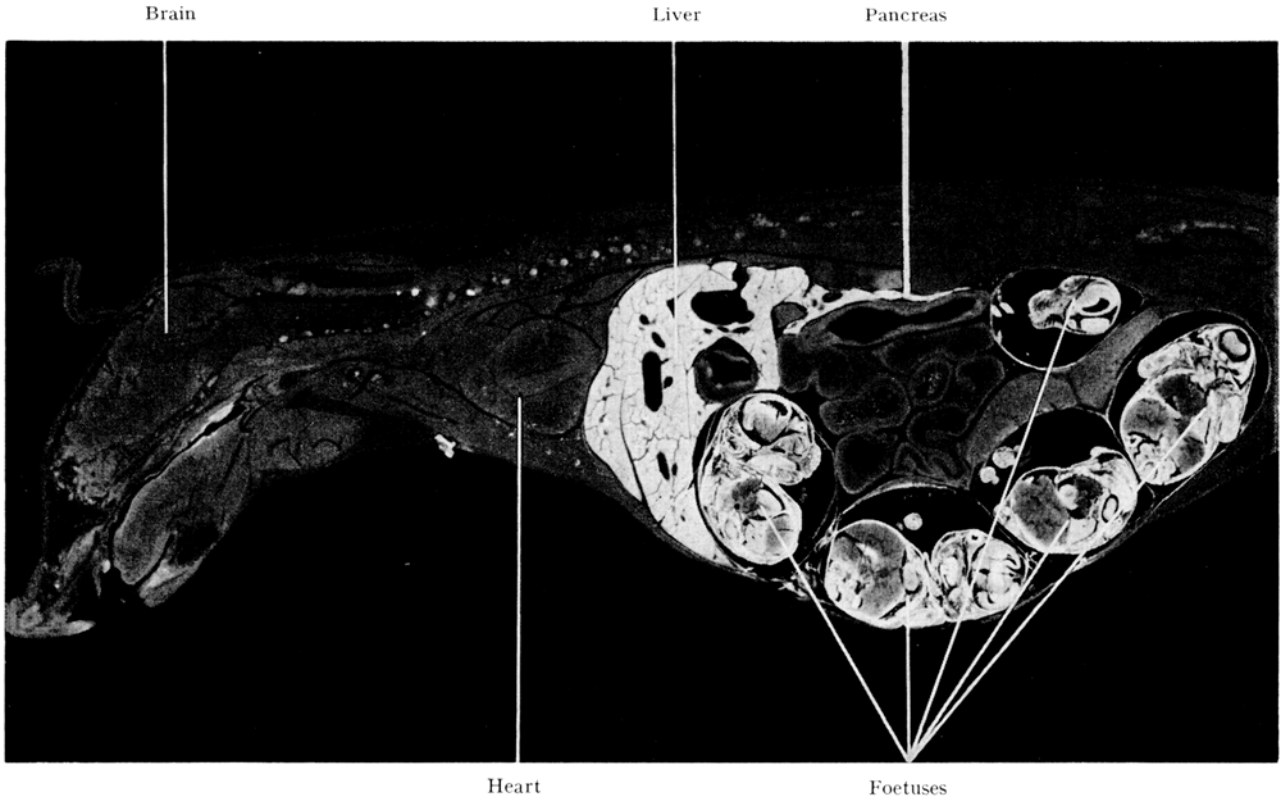
[2] C. M. Lee, B. A. Wiseman, S. A. Kaplan, and J. Warkany, J. Arch. Path. *59*, 232 (1955).

[3] C. A. Waddington and M. J. Perry, J. Embryol. exp. Morph. *6*, 366 (1958).

[4] H. Herrman, V. R. Konigsberg, and M. F. Curry, J. exp. Zool. *128*, 359 (1955).

[5] E. Farber and H. Popper, Proc. Soc. exp. Biol. Med. *74*, 838 (1958).

[6] R. C. Goldberg, I. L. Chaikoff, and H. Dodge, Proc. Soc. exp. Biol. Med. *74*, 869 (1950).



Autoradiogram showing distribution of radioactivity in a pregnant mouse 4 h after the intravenous injection of C^{14}-ethionine. The concentration in the foetuses is significantly higher than in any of the maternal tissues, except the liver and pancreas which have a concentration similar to that of the foetuses.

The mice were killed 5, 15, 30 min, 1, 4, 8, and 24 h after injection of ethionine and were subjected to autoradiography[7]. The autoradiographic studies show that ethionine rapidly penetrates the placenta of the mouse and accumulates in the foetus (Figure). High uptake can also be observed in the maternal pancreas and liver.

1 h after injection the concentration in the foetus is of the same magnitude as in the liver of the mother, but after 4 h the concentration in the foetus is greater than that in the mother.

In another series of experiments, the foetuses were removed 15 min and 1, 4, and 24 h after administration of the same amount of C^{14}-ethionine. The foetuses and the liver and pancreas of the mother were homogenized separately in 10% trichloroacetic acid (T.C.A.) and prepared as previously described[8]. Radioactivity of the T.C.A. soluble and of the T.C.A. precipitated and purified protein fraction was measured with a Tri-carb liquid scintillation counter[9].

Radioactivity was found only in the T.C.A. soluble fraction of the foetuses and of the maternal liver and pancreas, and no radioactivity could be observed in the protein fraction of the foetus and in the liver and the pancreas of the mother. The T.C.A. soluble radioactivity in the foetuses varied between 5.4–9.8% of the injected dose.

The experiments were repeated with C^{14}-*DL*-methionine (Amersham, spec. act. 6.6 mc/m*M*) and the results are shown in Table II. In contrast to the results obtained after injection of ethionine, a high incorporation was observed in the proteins of the pancreas, the liver, and the foetuses. For methionine, the radioactivity in the protein fractions (with the exception of the 15 min foetuses sample) was at least 5–10 times greater than in the T.C.A. soluble fraction.

In view of earlier findings on the incorporation of ethionine into animal tissue[10], and in order to find an explanation for the toxic effect on the foetuses, some further experiments were undertaken with higher doses of ethionine (5 μC). In these experiments radioactivity could be observed in the protein fraction but the main part of the radioactivity was found always in the T.C.A. soluble fraction (Table III).

Tab. I. Specific radioactivity in the T.C.A. (10% trichloroacetic acid) soluble fraction and in the protein fraction of foetus and material liver and pancreas after the intravenous injection of pregnant mice with C^{14}-ethionine.

Mouse No.	Time after administration of C^{14}-ethionine	T.C.A. soluble radioactivity Cpm/mg dry weight			Protein bound radioactivity Cpm/mg protein		
		foetus	liver	pancreas	foetus	liver	pancreas
1	15 min	78	58	697	0	0	0
2	1 h	154	30	281	0	0	0
3	4 h	290	120	322	0	0	0
4	24 h	76	56	274	0	0	0

[7] S. ULLBERG, Acta radiol. Suppl. 118 (1954).
[8] E. HANSSON, Acta physiol. scand. Suppl. 161 (1959).
[9] M. VAUGHAN, D. STEINBERG, and J. LOGAN, Science *126*, 446 (1957).
[10] L. LEVINE and H. TARVER, J. biol. Chem. *192*, 835 (1951).

Paper chromatography was performed after hydrolysis[11] of foetal proteins from the ethionine-injected animals and the radioactivity was determined on the chromatograms by means of autoradiography. The radioactivity was detected mainly in the spots corresponding to ethionine and ethionine sulphoxide.

Tab. II. Specific radioactivity in the T.C.A. (10% trichloroacetic acid) soluble fraction and in the protein fraction of foetus and maternal liver and pancreas after the intravenous injection of pregnant mice with C^{14}-methionine

Mouse No.	Time after administration of C^{14}-methionine	T.C.A. soluble radioactivity Cpm/mg tissue dry weight			Protein bound radioactivity Cpm/mg protein		
		foetus	liver	pancreas	foetus	liver	pancreas
5	15 min	27	31	311	40	215	780
6	1 h	32	22	66	165	187	519
7	4 h	26	23	24	329	191	215
8	24 h	55	13	32	144	141	114

Tab. III. Specific radioactivity in the T.C.A. (10% trichloroacetic acid) soluble fraction and in the protein fraction of foetus after the intravenous injection of pregnant mice with C^{14}-ethionine.

Mouse No.	Time after administration of C^{14}-ethionine	T.C.A. soluble radioactivity Cpm/mg tissue dry weight	Protein bound radioactivity Cpm/mg protein
9	15 min	109	8
10	15 min	1983	69
11	1 h	579	20
12	1 h	3045	60
13	4 h	643	20
14	4 h	994	59
15	24 h	4148	34
16	24 h	1163	74

These studies indicate that ethionine may freely pass the placenta barrier of the mouse and accumulate in the foetus. A very small part of the radioactive ethionine is incorporated into the foetal proteins. From this has been assumed that an abnormal protein is formed in which the naturally occurring amino acid has been replaced by the analogue. Such a replacement may in part explain the toxic effect of ethionine on the foetus. Most of the radioactivity, however, is found in the non-protein fraction, and earlier observations[12] have shown that ethionine can furnish ethyl radicals for synthesis of choline and thus produce triethylcholine which has proved to have a growth-inhibiting effect on rats. The present observations therefore support the concept that ethionine may exert its effect on the organism in other ways in addition to the formation of abnormal proteins.

Zusammenfassung. Trächtige Mäuse wurden zwei Tage vor dem erwarteten Werfen mit dem Antimetabolit des Methionins ^{14}C-*DL*-Aethionin intravenös behandelt. Aufnahme und Einbau des Antimetabolits wurden gleichzeitig autoradiographisch und chemisch untersucht. Die Plazentaschranke ist für Aethionin durchlässig; die Radioaktivität fand sich in den Foeten angehäuft, dabei war aber nur ein ganz geringer Teil des Aethionins in den Eiweisskörper derselben eingebaut. Der Einbau des Aethionins in den Organen (Leber und Pankreas) der Mutter war ebenfalls äusserst gering.

Aus den Ergebnissen wird der Mechanismus der toxischen Wirkung des Aethionins erklärt.

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Department of Pharmacology, Kungl. Veterinärhögskolan, Stockholm (Sweden), June 28, 1961.

[11] D. Gross and H. Tarver, J. biol. Chem. *217*, 169 (1955).
[12] J. A. Stekol and K. Weiss, J. biol. Chem. *185*, 577 (1950).
[13] Present address: Chemical Institute of the Medical University, Budapest (Hungary).

Mechanism of the Serotonin Depressor Response Following BAS-Phenol Administration in the Dog

The number of serotonin (5-hydroxytryptamine) antagonists which have been tested in both isolated tissues and intact animals is legion, but only a select few have been shown to be active against the cardiovascular effects of serotonin. The benzyl analog of serotonin, 1-benzyl-2-methyl-5-methoxytryptamine or BAS, is one of the more potent antagonists and it has been found to be effective against the pressor action of serotonin in the dog[1]. An even more powerful serotonin antagonist has been synthesized from BAS by cleavage of the methyl ether to form 1-benzyl-2-methyl-5-hydroxytryptamine or BAS-phenol[2]. In anesthetized dogs, intravenous injection of BAS-phenol blocks the pressor response to serotonin selectively while the pressor action of tryptamine remains unaffected, or may even be potentiated[3]. Possibly as a result of blockade of the pressor component of serotonin action, augmentation of the depressor phase in its activity occurs. The following studies were undertaken to investigate the mechanism of the depressor response to serotonin which is unmasked by the administration of BAS-phenol in the anesthetized dog.

Experiments were performed on 14 mongrel dogs of both sexes, weighing from 8 to 18 kg. The dogs were anesthetized with sodium pentobarbital[4], 30 mg/kg, administered intravenously. Arterial blood pressure was recorded with a mercury manometer from a femoral artery. Drug injections were made into the cannulated ipsilateral femoral vein. The agonist drugs and corresponding doses used were serotonin creatinine sulfate, 10–120 μg/kg, tryptamine hydrochloride, 125–500 μg/kg, histamine phosphate, 3–5 μg/kg, and acetylcholine bromide, 5–10 μg/kg. The antagonist drugs used were 1-benzyl-2-methyl-5-hydroxytryptamine hydrochloride (BAS-phenol)[5], 2.5 mg/kg, and diphenhydramine hydrochloride[6], 5 mg/kg. Doses for the agonist drugs are expressed in terms of the base while those for the antagonists are in terms of the salt.

[1] E. Shaw and D. W. Woolley, J. Pharmacol. *116*, 217 (1956).
[2] E. Shaw and D. W. Woolley, Proc. Soc. exp. Biol. Med. *96*, 439 (1957).
[3] D. W. Woolley and E. Shaw, J. Pharmacol. *121*, 13 (1957).
[4] Trade name Nembutal.
[5] Generously supplied by Dr. K. Pfister, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.
[6] Trade name Benadryl.