that the addition of methionine to a diet containing fibrin completely protected the rats.

Normal kidneys were found in 40 g rats fed the following diet for 10 days: casein, 15; salt mixture, 4; calcium carbonate, 1; codliver oil, 5; lard, 35; agar, 2; sucrose, 32, and yeast, 6. Hemorrhagic kidneys invariably occurred if 0.3% cystine was added but not if 0.1% choline was added in addition to the cystine.

Hemorrhagic kidneys resulted if the protein of the above basal ration consisted of fibrin, 4; casein, 8, and dried egg white, 3. The addition of 0.04% choline or of 1% dl-methionine completely protected the rats.

The ratio of the 2 amino acids, methionine and cystine, is not the only factor which determines the choline requirement. This became evident from the fact that hemorrhagic lesions were produced on the 15% casein diet by decreasing the level of choline in the diet through substitution of vitamin concentrates for the codliver oil and yeast and through lowering the fat content to 10%. Furthermore, the effect of a fibrin diet in producing the renal lesions was no longer evident if the fibrin was decreased from 15 to 5%. It is suggested that the absolute amount of either methionine or cystine, as well as the ratio of the 2, plays an important rôle in the interrelationship of these 2 amino acids and choline.

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Nitrogen-Containing Carcinogenic Compounds.

LIONEL JOSEPH. (Introduced by E. V. Cowdry.)

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Of all the heterocyclic nitrogen compounds studied, up to the present, none has been related to the carcinogenic hydrocarbons, such as methylcholanthrene, to the extent of containing an anthracene or phenanthrene nucleus. In view of the pronounced carcinogenic action of these hydrocarbons and in view of the fact that certain nitrogen compounds *not* related to these hydrocarbons have been found to be carcinogenic it seemed advisable to study the carcinogenic effect of heterocyclic nitrogen compounds related to these hydrocarbons to the extent of containing an anthracene or phenanthrene nucleus. Further, since certain indole derivatives which are not related to the car-

cinogenic hydrocarbons have been shown to be carcinogenic it seemed of interest to prepare an indole derivative which would be related to a carcinogenic hydrocarbon and to test its carcinogenic activity. For this purpose 2,9(N)-indoloanthone was prepared. This compound is readily reduced to the anthranol which is then analogous to a hydroxybenzpyrene. However the reduced form could not be obtained pure due to the rapid oxidation by atmospheric oxygen. Therefore the oxidized form of the compound was used in the hope that this might be reduced by the body tissues.

The other heterocyclic compounds studied were 1,2(N)-pyridinoanthracene, 1,2(N)-pyridinoanthracene methiodide, 3(N), 4-pyridinophenanthrene, and 3(N), 4-pyridinophenanthrene methiodide.

In addition it was decided to test 3-aminophenanthrene. This was done because of the observation made by Shear[1] that 2-aminoanthracene was capable of producing liver tumors and it was felt that 3-aminophenanthrene should be more effective than the corresponding anthracene derivative because, in general, phenanthrene derivatives are more efficient than anthracene derivatives.

2,9(N)-indoloanthrone was prepared by the method of Scholl.[2] Naphthalene was condensed with phthalic anhydride in the presence of aluminum chloride. Ring closure was effected by means of concentrated sulphuric acid and the resulting benzanthraquinone nitrated with a mixture of acetic anhydride and fuming nitric acid. The 2 nitro compounds were separated by fractional crystallization from chloroform and recrystallized from benzene. The 2-nitrobenzanthraquinone was treated with phenylhydrazine to give the desired compound.

Pyridinoanthracene was prepared by first reducing beta-aminoanthraquinone to beta-aminoanthracene according to the method of Braun and Bayer,[3] then treating the product with glycerin and concentrated sulphuric acid to yield the desired product, m.p. 169°C. The methiodide of this material was obtained by warming equivalent amounts of pyridinoanthracene and methyl iodide in 5 volumes of absolute alcohol for several minutes and recrystallizing the product from 95% alcohol; m.p. 221°C dec.

Pyridinophenanthrene was prepared according to the method of Mosettig and Krueger[4] by treating 3-aminophenanthrene with glycerine and sulphuric acid. The methiodide was prepared in the

[1] Shear, M. J., *Am. J. Cancer*, 1937, **29**, 269; *J. Biol. Chem.*, 1938, **108**, 123.

[2] Scholl, R., *Ber. d. Deut. Chem. Ges.*, 1911, **44**, 2370.

[3] Braun, J., and Bayer, O., *Ann. d. Chem.*, 1929, **116**, 472.

[4] Mosettig, E., and Krueger, J. W., *J. Am. Chem. Soc.*, 1936, **58**, 1311.

same manner as pyridinoanthracene methiodide; m.p. 239-240°C dec. The 3-aminophenanthrene used in this preparation was prepared from phenanthrene by the method of Bachmann and Boatner.[5]

A 2% solution of each of these compounds in benzene was painted twice weekly on the neck of 100 mice over a period of approximately 5 months. In addition to this .02 g of each of the compounds was suspended in 0.5 cc of paraffin and this dose was planted subcutaneously in 20 mice. These animals were observed for approximately 7 months. Neither in the instance of the skin paintings nor in those of the subcutaneous implantations did carcinoma occur.

Though certain polycyclic hydrocarbons of the benzanthracene type are carcinogenic, and despite the fact that certain nitrogen-containing compounds have also been found to be carcinogenic, these compounds, prepared and tested containing nitrogen and chemically analogous to benzanthracene, are probably non-carcinogenic. This observation serves to render our concept of carcinogenic substances more precise.

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Aldehydic Resorption in Mice.

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Kudrjashov and Agatov[1] reported that they were able to induce temporary sterilization, or failure of implantation, in female rats and rabbits by means of fractions obtained from rancid fat and oleic acid. The rancid fat did not act upon the sexual system of the rat, but caused the death of the embryo at or soon after implantation, the placental sign occurring on the 9-10th day (normal 13th day). The authors believe that the active substance may consist of aldehydes and ketones.

Strong's[2] very interesting observations on the liquefaction and regression of spontaneous mammary tumors by means of heptaldehyde have aroused considerable interest, and any light on the mechanism of heptaldehyde on tumors should be welcome at this time. He[3]

[5] Bachmann, W. E., and Boatner, C. H., *J. Am. Chem. Soc.*, 1936, **58**, 2097.

[1] Kudrjashov, B. A., and Agatov, P. A., *Ginekologia i Akusherstvo*, 1935, **6**, 1.

[2] Strong, L. C., *Am. J. Cancer*, 1939, **35**, 401.

[3] Strong, L. C., *Science*, 1938, **88**, 11.