of nucleic acid synthesis is particularly important in *Endamoeba histolytica*[5,6], the effect of azaserine on the amœbæ was studied.

The activity of azaserine was assayed in bacteria-free cultures of *E. histolytica* in a medium previously described . Azaserine was amœbacidal in concentrations of 1/10,000 (100 μgm./ml.)–1/20,000 (50 μgm./ml.). Adenine (100 μgm./ml.), adenylic acid (100 μgm./ml.) and 2,6-diaminopurine (100 μgm./ml.) completely reversed the inhibitory effects of azaserine. Serine, methionine, 4-amino-5-imidazolecarboxamide and leucovorin (tested singly and in various combinations) effected only a partial reversal of the toxic action of azaserine. Guanine, guanosine and guanylic acid were completely inactive in reversing the action of azaserine.

It seems that azaserine functions as an anti-metabolite in purine ring biosynthesis. Its mechanism of action against *E. histolytica* may be the prevention of 1-carbon incorporation as postulated[4,8] in the *de novo* synthesis of purines or the prevention of the closure of the acyclic purine ring precursor, thus blocking the synthesis of nucleic acids. Although 6-diazo-5-oxo-L-norleucine, another new tumour-inhibitory substance, was reported to inhibit the incorporation of formate into nucleic acids[8], no activity could be demonstrated against the amœbæ.

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Riboflavin in Milk

Such studies as have been made on the riboflavin in milk point to the existence of species differences in respect of the forms in which this vitamin occurs in milk. Thus Kon and Mawson[1] reported that the concentration of riboflavin in human milk is very low, and found that the method of Emmerie[2], in which riboflavin is extracted by methanol was inapplicable to it. Davis *et al.*[3] found the methanol method unsuitable for sow's milk, and at the same time suggested that riboflavin might be present in sow's milk in a form different from that occurring in cow's milk. In view of the foregoing observations, the partition of riboflavin between free riboflavin, flavin mononucleotide and flavin adenine dinucleotide in the milk of several species has been studied.

Riboflavin was extracted from milk by the method of Bessey *et al.*[4]. From the neutralized extract the riboflavin was extracted with liquid phenol. Addition of ether threw out an aqueous phase containing all the riboflavin. Both by paper chromatography and by paper electrophoresis, free riboflavin, flavin mononucleotide and flavin adenine dinucleotide were separated. For the chromatographic separation a butanol/acetic acid/water system was used (Crammer[5]). For the paper electrophoresis a borate buffer (0·05 *M* borax, *p*H 9·2) was used, and a potential of 300 volts was applied for 5 hr. giving a current of 1·5 m.amp. per cm. width. Distinctly separated spots were made visible by ultra-violet illumination, and known samples of free riboflavin, flavin mononucleotide and flavin adenine dinucleotide were used for their identification. R_F values of the flavins for the solvent system used in the chromatography were 0·04 for flavin adenine dinucleotide, 0·13 for flavin mononucleotide and 0·35 for free riboflavin.

With the milk of cows, goats, ewes and rabbits two spots were observed, the larger one corresponding to free riboflavin and the smaller one to flavin adenine dinucleotide. On the other hand, with human, mare's and sow's milks usually only one spot was obtained, which corresponded to flavin adenine dinucleotide. Occasionally, however, with these last three types of milk, faint spots corresponding to flavin mononucleotide and free riboflavin were observed, but they probably resulted from slight hydrolysis of the flavin adenine dinucleotide. The authenticity of the flavin adenine dinucleotide was always established by enzymatic assay in the D-amino-acid oxidase system.

The ultrafiltrates from cow's and goat's milk were yellow, and under ultra-violet light they showed the characteristic greenish fluorescence of free riboflavin, whereas the ultrafiltrates of human and mare's milk were colourless. None of the ultrafiltrates contained flavin adenine dinucleotide, which suggests either that this molecule was too large to pass through the filter membrane or that it was present in the milks in a bound form.

Since in human, mare's and sow's milks the riboflavin is present as flavin adenine dinucleotide it might be thought that these milks, compared with cow's, goat's, ewe's and rabbit's milks, would contain greater amounts of enzymes, such as xanthine oxidase, that have flavin adenine dinucleotide as their prosthetic group. However, preliminary experiments have shown that xanthine oxidase is absent from human, mare's and sow's milks but is present in the milks of the other four species. This work is still in progress.

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