Changes in Serum-Protein Levels in Rabbits Treated with 1:2:5:6 Dibenzanthracene

NUMEROUS attempts have been made to demonstrate specific changes in blood proteins in direct relation to malignancy. For a summary of the literature on this subject, the reader is referred to the work of Stern and Wilheim[1], and to the more recent article by A. B. Gutman[2]. Most of these studies have been made on animal and clinical material with a fully developed picture of malignancy.

In a research designed to investigate the nature of the changes in blood proteins in malignancy, we have conducted a series of preliminary studies, the object of which was to follow any deviation in serum-protein levels from the normal through the precancerous state to final malignancy.

Adult male rabbits were employed as experimental animals, using the carcinogen 1 : 2 : 5 : 6 dibenzanthracene. The investigations extended over a period of six months, when they were terminated owing to the death of three of the eight treated animals; four animals were maintained in the control series. Blood was obtained by simple venipuncture of the ear, and weekly determinations were made of the total serum proteins, total albumin and total globulin, employing the biuret reaction[3]; body-weights of both the control and treated animals were also determined at weekly intervals for the entire period of the experiment.

After determining the serum-protein levels for one month to obtain the normal level for each animal, 200 mgm. of 1 : 2 : 5 : 6 dibenzanthracene, contained in a gelatine capsule, were surgically implanted into the abdominal wall of each of the eight experimental animals. At the end of six months, all the surviving animals were sacrificed and necropsies performed. Three animals were found to have bile duct carcinomas, a fourth a carcinoma of the lung, and a fifth a spindle cell sarcoma at the site of implantation of the dibenzanthracene. The remaining three experimental rabbits were free from malignancy, and appeared to have no liver involvement.

Determinations of the total serum proteins showed no significant differences in levels between the treated and control series. With the exception of one animal suffering from a tumour of the liver, and showing a lowered serum albumin content, no differences were also observed in the levels of the albumin fraction. However, in the case of the serum globulin this was found to be significantly increased in all animals with liver tumours; there was no increase in this fraction in any of the other tumour-bearing animals; experimental animals which did not develop tumours showed no significant differences in any of the protein determinations made.

From the experimental data so far available, it would appear that 1 : 2 : 5 : 6 dibenzanthracene does not produce a significant change in the levels of any of the protein fractions so far examined, with the exception of animals that develop tumours of the liver, when there is a rise in the globulin fraction. We conclude, therefore, that if any change does occur, it does so within one or more of the individual fractions of these proteins; these possibilities are being investigated at the present time, and preliminary results on animals treated with 20-methyl cholanthrene sixteen weeks ago, and now presumably in a precancerous state, suggest an increase in the protein fraction precipitated by 25 per cent saturation with ammonium sulphate, and a further increase in the fraction not precipitated by 70 per cent saturation with ammonium sulphate, but precipitated by 10 per cent trichloracetic acid; whether or not these changes will continue and persist in malignancy remains to be determined.

Our thanks are due to Mr. J. H. Thompson, director of research at these Laboratories, to Dr. G. J. W. Ollerenshaw for the surgical implantation of the dibenzanthracene, and to Dr. R. Fleming for the preparation of numerous sections.

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Feb. 18.

[1] Stern, K., and Wilheim, R., "Biochemistry of Malignant Tumours", 109 (Chem. Pub. Co., Inc., Brooklyn, N.Y., 1943).
[2] Gutman, A. B., "Advances in Protein Chemistry, 4, "The Plasma Proteins in Disease" (Academic Press, Inc., N.Y.).
[3] Robinson and Hogden, *J. Biol. Chem.*, **135**, 707, 727 (1940).

Drug Action of Formaldehyde Derivatives of Sulphathiazole

THE present-day concept of effective sulphonamide therapy is based on experimental evidence which conditions high concentration in extracellular fluid and a minimum of localization in tissue cells as essential desiderata for successful drug action. Our experiments with the formaldehyde derivatives of 2-sulphanilamidothiazole, against a Gram-positive coccus (*Streptococcus pyogenes*), a Gram-negative bacillus (*Salmonella typhi*) and a vibrio (*Vibrio cholerœ*) indicate an essentially different mechanism of drug action.

In a previous communication[1] reference was made to (1) the preparation by one of us (S. S. B.) of compounds by hexamethylene-tetra-amine linkage to *p*-aminobenzene-sulphanilamide, and (2) the condensation of formaldehyde with sulphathiazole by Meier[2] and Druey[3], which, in our hands, proved to be an effective antidote for the prevention and treatment of Asiatic cholera[4]. Further studies on experimental infections with the typhoid bacillus and the streptococcus presented the puzzling observation that potent chemotherapeutic activity went along with a peculiarly prolonged low blood concentration, a fact which did not fit in with the accepted concept of 7–10 mgm. per cent blood value for effective therapeutic action.

Since formosulphathiazole is only slightly soluble in water and common organic solvents, its therapeutically active sodium salt was prepared by interaction with sodium carbonate, which gave an alkaline product reacting in the N–1 position with the hydrogen of different sulphamyl groups. At the same time this reaction indicated that the condensat on of formaldehyde with sulphathiazole takes place on a 3 mol. : 3 mol. basis, giving rise to a compound of unusually high molecular weight.

Metabolism of the parent compound and its sodium salt was studied in mice, guinea pigs, rabbits and dogs by colorimetric determination of blood values, urinary excretion and tissue concentration, in terms of sulphathiazole standards, by a modified method which combined the Bratton and Marshall[5] and Druey and Oesterheld[6] techniques. The plasma-water concentration ranged only between 1–2 mgm. per cent in spite of the maximum tolerated dose and parenteral route employed. This was also the case