

The present experiments attempt to increase the plasma protein level in rats fed an azo dye and to determine the effect upon the incidence and rate of liver tumor formation.

Materials and methods. Rats with developing liver tumors were united in parabiosis with normal rats, or they were united on each side to normal rats as parabiotic triplets. The normal rats might be expected to provide plasma proteins to the pre-tumorous rats by way of the common vascular connections. Fluid restriction, during and after feeding of the dye, was also employed with some of the rats. Serum density determinations were made according to the method of Van Slyke described in TÁRNOKY⁴. The rats were fed 3'-methyl-4-dimethylamino-azo-benzene for three months at a 0.06% level in the synthetic diet developed by GELBOIN, MILLER and MILLER⁵. Control rats of the same weight were fed the synthetic diet without the carcinogenic azo dye. Parabiosis was carried out according to the method of HILL⁶.

Results. Parabiosis. The average survival period of 21 dye-fed control rats was 5.5 weeks after the cessation of dye feeding. Twelve dye-fed rats were united in parabiosis with single control rats; the average survival time was 7 weeks after termination of feeding of the dye. Control rats which had been in parabiotic union with tumorous rats until their death were separated surgically and kept under observation. Laparotomies upon these rats did not reveal any sign of liver damage or tumor formation 2.5 months after separation from the tumorous rats. In eight cases dye-fed rats were united between two normal rats as parabiotic triplets. The average survival time of the tumorous rats was 10.8 weeks after dye feeding was discontinued.

Fluid restriction. Ten rats were subjected to five day periods of fluid restriction during the three months the dye was fed and for 1½ months after dye feeding. Ten other rats of a similar age were given water *ad libitum* but the consumption of the synthetic diet containing the azo dye, and later the rat chow, of each of these rats was limited so that it was similar to that of the fluid restricted rats. Another group of ten rats had access to the diet and water at all times. 1½ months after the dye feeding period the average weight of the fluid restricted rats was 184 g, that of the pair-fed controls was 178 g and the average weight of the rats with unlimited food and water supply was 340 g. All the rats were sacrificed at this time and it was found that none of the fluid restricted or pair fed rats showed any sign of liver damage or tumors while the group without fluid or food restriction all showed varying degrees of liver tumor development.

Before the above groups of rats were sacrificed serum density determinations⁴ were performed on the rats of the fluid and food restricted groups, as well as upon five normal rats and five normal rats that had been subjected to two seven day periods of water restriction. The average serum density of both types of normal rats and the fluid restricted rats was 1.029 or 8% serum protein, whereas the average serum density of the food restricted rats was 1.027 or 7.5% serum protein.

Discussion. In the present experiments parabiosis (with one or two normal rats) slightly retards the average rate of tumor development in rats which had been fed the azo dye, but does not prevent their eventual death from liver tumors. Since the fluid restriction experiments demonstrated that tumor development could be inhibited by decreasing the food consumption after the period of dye feeding, it seems probable that the slight retardation in tumor development due to parabiosis is attributable to decreased food consumption by the dye-fed rats because of the difficulties of feeding while in the parabiotic association.

Prolonged periods of fluid restriction were found to inhibit liver tumor formation in rats fed the azo dye. However pair-feeding experiments indicated that tumor development was inhibited due to the decreased food consumption of these rats. This conclusion is further supported by the finding that fluid restriction did not increase the relative concentration of serum proteins. TANNENBAUM and SILVERSTONE⁷ have previously found that the promotional, but not the initiation, of carcinogenesis can be inhibited by caloric restriction.

Résumé. Chez le rat, le développement des tumeurs du foie est ralenti lorsqu'on met les individus atteints en parabiose avec des individus normaux et arrêté si l'on réduit leur ration d'eau. Mais il semble que dans les deux cas, ces effets ont pour cause déterminante la diminution de la nourriture consommée.

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⁴ A. TÁRNOKY, *Clinical Biochemical Methods* (Hilger and Watts Ltd., London 1958).

⁵ H. V. GELBOIN, J. A. MILLER, and E. C. MILLER, *Cancer Res.* 18, 608 (1958).

⁶ R. T. HILL, *J. exp. Zool.* 63, 203 (1932).

⁷ A. TANNENBAUM and H. SILVERSTONE, *Adv. in Cancer Res.* 1, 451 (1953).

Human Vascular Antigen Complement Consumption Test of Hypertensive Patients (Preliminary Report)

Owing to its high incidence throughout the world, hypertensive vascular disease is constantly in the focus of interest. Recent research has greatly enhanced our knowledge of the condition, but the pathogenesis has still many obscure features.

We have undertaken to study vascular lesions in hypertensive patients, with special reference to the potential role of autoaggressive processes. To elucidate the problem, complement consumption tests have been made with vascular antigens, thus modifying the 'leucocyte complement consumption test' of CHUDOMEL, JEZKOVA, and LIBÁNSKY¹ so that it became suitable for the demonstration of binding of vascular antigen-autoantibodies.

Methods. Specimens of arteries and aorta were taken from various parts of blood group 'O' hypertensive cadavers, homogenized in 'Ultra-turax' and stored at -10°C. To titrated amounts of the homogenate, active test sera were added and the mixtures were incubated at 37°C. After 1 h of incubation and centrifugation, the sera were tested for complement content. A decrease of serum complement results (as compared with the serum complement level in the absence of vascular antigen) if a vascular antigen-autoantibody union has taken place. Corresponding to the measure of the fall in complement titre, we speak about a '+' reaction (3 tubes), '++' (5 tubes) and '+++ reaction (5-10 tubes). A difference of 0-2 tubes may be accepted as negative. According to CHUDOMEL, JEZKOVA, and LIBÁNSKY¹, the method is equally suitable for the demonstration of complete and incomplete antibodies.

Results. The complement consumption tests yielded positive results in 60 out of 122 cases of hypertension, equivalent to 49.1% of all cases. In 3 cases, the sera were

¹ V. CHUDOMEL, Z. JEZKOVA, and J. LIBÁNSKY, *Blood* 14, 920 (1959).

	Number of patients examined	Number of positive results by the complement consumption test	Number of negative results by the complement consumption test	Number of anti-complementary sera
Sera from normal individuals	41	3	38	—
Sera from patients with various internal diseases	44	7	37	—
Sera from patients with hypertensive disease	122	60	59	3

anticomplementary. The controls were normal males and females, with normal blood pressure. Of the 41 normal controls, 3 showed positive test (7.3%) as compared with 7 out of 44 patients suffering from various internal diseases (16.1%). Thus, the results indicate that, although not specific, the vascular antigen complement consumption test employed by us was more often positive in the cases of hypertension than in the normal controls or among the patients with various internal diseases. The complement fixation tests involving the use of tissue antigens, the non-specific reactions that may occur, the difficulties that may be encountered, as well as the theoretical considerations, have been discussed in detail by BAKOS, SCHULHOF, SZILÁRD, and VAJDA², who showed that a positive complement fixation with tissue antigens was more common among patients with polyarthritis than among those suffering from various internal disorders, and that in some cases also an organ-specificity should be taken into account.

The mechanism of the positive complement consumption tests in cases of hypertension is still unclear. It appears that the binding of an autoantigen-autoantibody is involved. This view is supported by the earlier experiments of GORECZKY, RÓTH, SÁRFY, and SÜMEGI³, who showed that in animals the pathomorphological changes of periarteritis nodosa could be produced by treatment with arteriotoxic serum. These data were confirmed years later by STEFANINI⁴. SZIGETI and JÁKÓ⁵ demonstrated, by the antihuman globulin consumption method of STEFFEN⁶, the presence of incomplete antibodies in the sera from rabbits immunized with homologous vascular wall antigen. It was remarkable in the clinical evaluation of our positive cases that most of the patients had shown the symptoms and signs of renal lesion. Autoaggression is well-known to play a role in renal diseases (CAVELTI⁷, FRICK⁸, SARRE and ROTHER⁹, VORLAENDER¹⁰, WENDLBERGER and FRÖHLICH¹¹, FRÖHLICH and PRESSINGER¹²).

In the light of these data from the literature, the results of the vascular antigen complement consumption tests, as well as the presence of vascular lesions in patients with hypertension, call attention to a potential role of autoaggression in a certain percentage of such cases. It is still unknown how these processes of autoaggressive nature begin and how they influence hypertension.

Zusammenfassung. Der «Gefässantigentest» (complement-consumption-test) wurde bei 122 hypertensischen Kranken, bei 44 verschiedenen internen Patienten und bei 41 gesunden Personen durchgeführt. Das Ergebnis war bei den Hypertonikern in 49,1%, bei den Internen in 16,1% und bei den Gesunden in 7,3% positiv. Das Verfahren

kann aber nicht als spezifisch betrachtet werden, obwohl bei den Hypertonikern das Ergebnis öfters als bei anderen Patienten positiv ist.

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Development of Digits from the Proximal Pre-Axial Material of the Wing and Hind-Limb Bud in Chick Embryos

The epidermis plus a fairly thick layer of the underlying mesenchyme removed from the proximal two-thirds of the pre-axial border of one limb bud (St. 22 to 25 chicken embryos) was transplanted on a shallow incision of the distal third of the dorsal aspect of the same (A) or of the heteronomous (B) limb bud. The graft lay parallel to the preserved apical ridge of the host bud (Fig. *inset*). At the time of the operation the structure of the epidermis of the graft is similar to that of the epithelial covering of the dorsal and ventral surfaces of the limb bud; a small part of the *apical ridge* proper is present only in the grafts taken from stage 22 not from stage 23 to 25 embryos.

In the normal development *in situ*, the material which is transplanted in these experiments would give rise to a part of the territory of the arm and forearm (and respectively of the thigh and leg). Actually, from one to three supernumerary digits developed from the heterotopically grafted material of the pre-axial border. The characters of these digits are in the great majority of the cases typical of the donor limb (Fig.).

The results mentioned bear some resemblance to those obtained by implanting small isolates from the thigh mesoderm under the *apical ridge* of the wing bud (SAUNDERS et al.¹), or by transplanting a semilunar pre-axial portion of the wing bud over the proximal stump of the hind-limb bud (Case CH, St. 22, HAMPÉ²). However, in our experiments, the heterotopically grafted mesenchymal precursor of the arm and forearm (and respectively of the thigh and leg) was not in contact with the normal apical ridge of the limb bud; yet, terminal structures of the wing (and leg) developed in more than 90% of the grafts. Conversely, when the proximal portion of the pre-axial material was grafted to the dorsal aspect of the wing (or hind-limb) bud, but near to its base, or to the somite region, formation of digits did not occur.

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