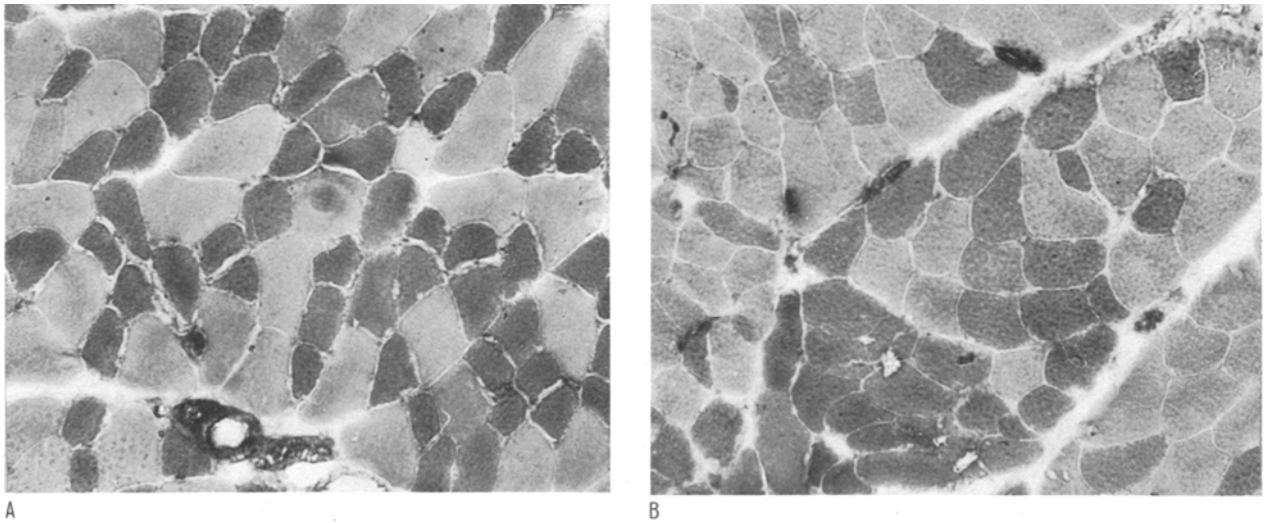

A B

A) Normal rat extensor digitorum longus muscle stained for ATPase.
B) 60-day sliced extensor digitorum longus graft stained for ATPase. Distinct differences in fibre type staining are seen. Fibre type grouping rather than the normal checkerboard pattern is common.

grafts are relatively slow at first and speed up as the regenerating muscle fibres mature.

Tetanic tensions of sliced grafts were consistently less than those of intact grafts throughout the developmental period tested (Table II). These differences are a reflection of the gross appearance of the two types of grafts. Grafts of intact muscles are normally thicker and have a larger area of densely packed muscle fibres than do sliced grafts. Comparisons of weights are of little value because of variable amounts of connective tissue adherent to the muscles.

Histochemistry. The histochemical differentiation of muscle fibres within the sliced grafts followed a course typical of that seen in regenerating mammalian muscle. By 30 days differences in fibre types were beginning to emerge when sections were stained for either SDH or ATPase activity, and by 60 days the grafts were populated with distinct type I and type II fibres (Figure). As is the rule in regenerated and transplanted muscles, type grouping was commonly observed. Overall, the histochemical preparations revealed a close return of the regenerated muscle fibres to the normal condition.

Discussion. The method of transverse slicing has proven to be a useful model for studying regenerating mammalian muscle, and it represents another technique whereby an entire muscle can be regenerated or transplanted. Slicing is superior to mincing with respect to the greater amount of functional muscle that regenerates. Sliced muscle grafts are easier to analyze physiologically and structurally than grafts of intact muscles. Because original muscle fibres do not survive, the developmental gradients of contraction can be assumed to represent the properties of regenerating muscle fibres alone. The strength of contraction, however, is less than that of intact muscle grafts. This is probably due to the disruption of the internal architectural framework of the muscle by the slicing process. Thus as a physiological model slicing is a preferable technique of muscle grafting whereas intact muscle grafts produce muscles that are better from the clinical standpoint.

Summary. Transverse slicing is a new technique whereby a mammalian muscle can be freely grafted with success. This method eliminates contamination of the early graft by surviving muscle fibres and allows one to measure the development of contractile properties on a uniform population of regenerating muscle fibres.

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[9] Part of this research was carried out while on a scientific exchange between the US and Czechoslovak Academies of Sciences.

Glutamine Aminotransferase and Glutamine Aminohydrolase Ratio as a Possible Test for Antitumour Compounds

CHAKRABORTY et al.[1], during their studies on the plasma ratio of glutamine aminotransferase (GAT) and glutamine aminohydrolase (GNase) in patients suffering from myeloproliferative diseases and different types of other blood disorders, had observed fluctuation in the ratio of these two enzymes with clinical and haematological conditions of the patients. They also found that the ratio was dependant upon the response of the patient to a particular drug therapy. Hence it was decided to see if the fluctuations in the plasma GAT/GNase ratio could be used as a test for the antitumour compounds. With this idea in view 3 of the known antitumour compounds were screened for their effect on the ratio of the two types

[1] P. CHAKRABORTY, M. DUTTA CHAWDHURI and G. C. SHRIVASTAVA, Indian. J. exp. Biol. in press (1974).

of glutaminases in the Ehrlich ascites cells and the host plasma.

Materials and methods. The Ehrlich ascites cells (EAC) were maintained in our laboratory by serial transplantation into Swiss mice. The processing of the Ehrlich ascites cells and plasma was carried out according to the method described by CHAKRABORTY and SHRIVASTAVA[2]. The incubation procedures and mixtures were similar to those described earlier[3]. The ammonia liberated during the incubation was estimated according to BRAGANCA et al.[4]. All antitumour compounds used were injected i.p. 10 days after the tumour transplantation.

Mitomycin-C manufactured by M/s. Kyowa Hakko Kgyo Company Ltd., Japan, was received as a gift from M/s. Biochemical Pharmaceutical Industries, Bombay, and Endoxan-Asta was supplied as gift by M/s. Khandelwal Laboratories, Bombay. The authors are thankful to both the firms for their free gifts. Actinomycin-D was obtained from M/s. Sigma Chemical Company, St. Louis, Mo., USA.

Results. Mitomycin-C was injected in normal and tumour-bearing mice at a concentration of 4 mg/kg b.wt. It can be seen from the Figure 1 that the injection of this antibiotic did not alter the plasma GAT/GNase ratio of 1.0 in normal mice, but there was a shift in the Ehrlich ascites cell ratio from 0.5 to 1.0 in about 6 h after the injection. However, it took a longer period for the plasma ratio to come up to the normal value. The actual time taken being 6 h more than the cells.

Similar studies were carried out with actinomycin-D which was injected at a concentration of 100 γ/kg b.w.t. and the results obtained show that this compound also behaved like the previous one, i.e. there was no shift in the GAT/GNase ratio in the normal mice, but the tumour cells and host plasma was elevated to the normal value of 1.0 in 6 and 12 h respectively (Figure 2).

20 mg/kg b.w.t. of Endoxan-Asta was injected into normal and tumour-bearing mice; its effect on the GAT/GNase ratio are depicted on Figure 3. Although Endoxan did not affect the ratio of the two enzymes in the plasma of normal animals, yet there was an effect on the tumour cells and host plasma values. It may be observed that the time taken for the ratio in the tumour cells and host plasma to come to normal value was much longer than those observed for the 2 previous antitumour compounds described above.

Discussion. From the above data it could be seen that all the 3 antitumour compounds affected the GAT/GNase ratio in the tumour cells as well as host plasma, but not the plasma obtained from the normal mice. It may be mentioned here that, with the return of GAT/GNase ratio to the normal value of 1.0, the survival time of the tumour injected animals was also increased. Lengthening of the life span of the tumour-bearing animals might indicate some sort of a correlation between the GAT/GNase ratio and the effect of antitumour agents. This is further substantiated by the fact that the peritoneal fluid of the drug-injected animals gave much less packed cell volume than that of ones which had no treatment.

The probable reason for the late effect of Endoxan-Asta could be the fact that this compound has to be transported to the liver where it gets activated in the presence of some microsomal enzyme system. For this activation oxygen and NADPH are used and then this activated compound is further activated at the site of the action. This activated derivative of Endoxan-Asta is supposed to interfere with cellular division at some vulnerable phase.

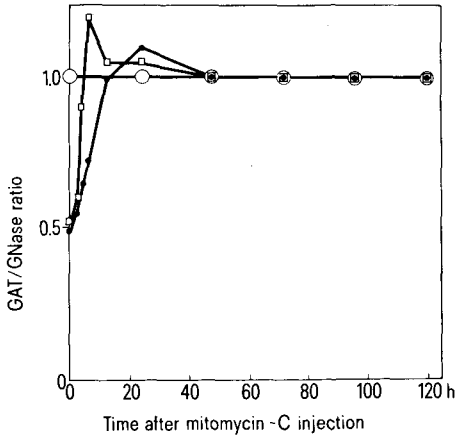

Fig. 1. GAT/GNase ratio after 4 mg/kg body weight injection of mitomycin-C. -○-, Plasma (normal animal); -□-, Ehrlich cells; -△-, Plasma (tumour-bearing animals).

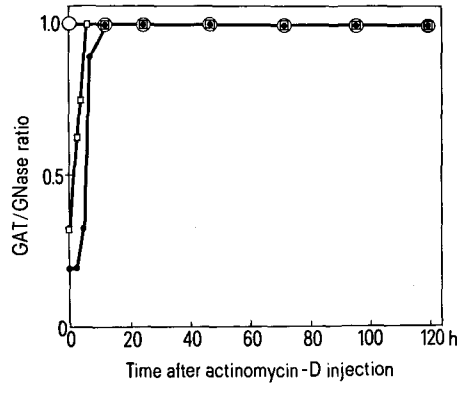

Fig. 2. GAT/GNase ratio after 100 γ/kg b.wt. injection of actinomycin-D. -○-, Plasma (normal animals); -□-, Ehrlich cells; -△-, Plasma (tumour-bearing animals).

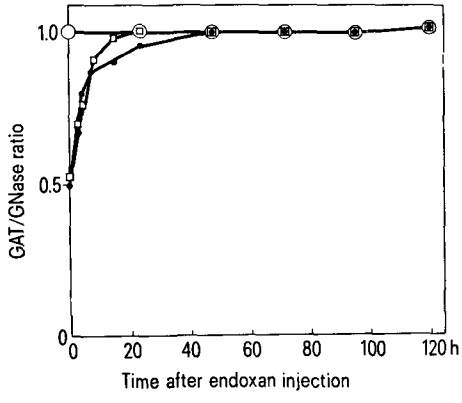

Fig. 3. GAT/GNase ratio after 20 mg/kg b.wt. injection of Endoxan-Asta. -○-, Plasma (normal animals); -□-, Ehrlich cells; -△-, Plasma (tumour-bearing animals).

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On the other hand, SHIBA et al.[5] found that mitomycin-C selectively inhibited the synthesis of DNA. It is also known that actinomycin-D inhibits the formation of mRNA from DNA. Thus both these compounds have a direct effect on the cells, hence the change in the GAT/GNase ratio is seen earlier than that with Endoxan-Asta.

In conclusion it may be said that the change in the plasma GAT/GNase ratio might possibly be useful for the testing of compounds which may have antitumour activity.

Summary. Three known antitumour drugs have been tested for their effect on the GAT/GNase ratio of Ehrlich Ascites cells and host plasma. It was observed that all these drugs had changed the ratio of the 2 types of glutaminases from below 1.0 to the normal value of 1.0, this was accompanied with an increase in the survival time of the tumour-bearing animals. There was, however, no effect on the plasma GAT/GNase ratio of normal animals in the presence of the 3 antitumour compounds tested.

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7 October 1974.

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[6] Acknowledgment. One of the authors (PC) is thankful to the Council of Scientific and Industrial Research, New Delhi, India, for an award of a Research Fellowship.

Are there Somatostatin-Containing Nerves in the Rat Gut? Immunohistochemical Evidence for a New Type of Peripheral Nerves

It has been generally accepted that the gut receives a dual innervation by cholinergic and adrenergic nerves, exerting, respectively, an excitatory and inhibitory effect in the control of gut motility[1-4]. More recently BURNSTOCK and collaborators have advanced the concept of a purinergic innervation to represent the main inhibitory system antagonistic to the excitatory cholinergic nerves (see[5]). Finally, immunohistochemical studies by NILSSON et al.[5] have demonstrated that, in the mouse, Substance P, or a Substance P-like substance, is present in nerves forming a dense network mainly around the ganglion cells of the myenteric plexus. Thus, at least part of the Substance P present in the gut[6,7] and originally isolated from this tissue by VON EULER and GADDUM[6] is of neuronal origin.

In the present paper we report the existence of probable nerves containing somatostatin or somatostatin-like immunoreactivity in the rat gut. This peptide was originally isolated from the hypothalamus and has an inhibitory action on the growth hormone release[8]. However, somatostatin is present not only in the hypothalamus[9-11], but has recently also been found in various peripheral tissues with immunohistochemistry[12] and radioimmunoassay[13].

Material and methods. Antibodies to somatostatin were prepared by coupling synthetic somatostatin to human α-globulin as described previously[14].

Male albino rats (6, wt. 150–200 g) were perfused with ice-cold 4% formalin, prepared according to PEASE[15] as described previously[16]. After rinsing, cryostat sections were cut from the stomach, duodenum, jejunum, ileum, colon and rectum. The sections were incubated with somatostatin antiserum pretreated with human α-globulin, diluted 1:20, for 30 min, rinsed in phosphate buffered saline (PBS), incubated with fluoresceinisothiocyante conjugated sheep antirabbit immunoglobulin (Statens Bakteriologiska Laboratorium, Stockholm, Sweden), diluted 1:4 for 30 min, rinsed in PBS, mounted and examined in a Zeiss Junior fluorescence microscope. Consecutive sections were incubated with somatostatin antiserum adsorbed with somatostatin (control serum) or with Substance P. All sera contained 0.3% Triton X-100[17].

Results and discussion. After incubation with somatostatin antiserum pretreated with human α-globulin, principally two types of structures exhibited a positive immunofluorescence. Firstly, cells mainly localized in the lamina propria of the gut mucosa but partly also among the gland cells were green fluorescent. However, except in certain cases described below, cells with a similar localization were immunopositive not only after control serum followed by FITC conjugated antibodies but also after incubation with FITC conjugated antibodies alone. Thus, many, but probably not all, fluorescent cells seen after incubation with somatostatin antiserum cannot be considered as containing somatostatin. On the other hand, in the stomach morphologically characteristic cells, mostly sending out short, thick processes, were observed only after incubation with somatostatin antiserum, but not in the controls. These cells may thus represent true somatostatin-containing cells confirming the radioimmunological results of ARIMURA et al.[13]. The localization of somatostatin in cell bodies in the intestinal wall will be discussed in detail in a following paper[18].

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