Hemmstoffe des Trypsins, Plasmins und Thrombins beschrieben worden[12,13,19,20]. Die Reaktion von GBNB mit Urokinase erfolgt mit geringerer Geschwindigkeit als mit Trypsin und Plasmin[12]. Nachdem bereits von anderen Autoren gezeigt worden ist, dass der irreversible Chymotrypsininhibitor N^{α}-Tosyl-L-phenylalaninchlormethylketon (TPCK) nicht mit Urokinase reagiert[3], wurde nunmehr festgestellt, dass auch TLCK keine Hemmung des Fermentes bewirkt. Da eine Hemmung anderer plasminogenaktivierender Fermente durch TLCK nachgewiesen wurde[21], deutet dieses Ergebnis auf bestehende Unterschiede in den aktiven Zentren derartiger Fermente hin.

Summary. Urokinase, the plasminogen activator from urine, is inhibited irreversibly by diisopropyl fluorophosphate and 4′-nitrobenzyl 4-guanidinobenzoate. The reactions of inhibition underlie pseudofirst-order kinetics. Rate constants were estimated. Urokinase is not inactivated by incubation with tosyl-L-lysine chloromethyl ketone.

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A Dialysable Glutamine Hydrolysing Enzyme from Normal and Malignant Tissues

A previous report[1] had indicated the presence of a dialysable factor in the Ehrlich ascites cells, which could bring about a degradation of glutamine in vitro. It was, therefore, of interest to see if a similar phenomenon occurred in solid tumours also and compare it with kidney, a rich source of glutaminase. An attempt has also been made to determine the nature of the factor, obtained after dialysis.

Materials and methods. The animals, bearing a mouse fibro-sarcoma (MFS), described by Waravdekar et al.[2], were killed 10 days after the tumour transplantation, by cervical dislocation. A 20% homogenate of the tumour tissue was prepared in cold calcium, free Krebs-Ringer solution. Kidney homogenates were also prepared in a similar manner. 5.0 ml of homogenates were dialysed against 30 ml of calcium, free Krebs-Ringer phosphate buffer, pH 7.4 at 4 °C for 18 h. The dialysates were then lyophilized and reconstituted with water just before use.

All incubations were carried out in Ca-free Krebs-Ringer phosphate buffer, pH 7.4, containing 5 m*M* glutamine, in a conventional Warburg manometric apparatus at 37 °C for 1 h. The ammonia produced was determined by the method of Braganca et al.[3]. The results obtained were corrected for the non-enzymatic hydrolysis of the substrate due to the addition of K_2CO_3.

Protein determinations were carried out according to the method of Sutherland et al.[4].

Results. Table I shows that both the dialysates and the dialysed homogenates, obtained from tumour and kidney, can liberate ammonia from glutamine. The activity, however, was higher in the former than in the latter. As the dialysates also showed the presence of protein, we tried to determine the heat stability of this factor, by keeping the dialysates in boiling water for 10 min and then tested for their activities. Table I indicates that, whereas the kidney dialysate lost about 89% of its original activity, the tumour dialysate was inactivated to the time of about 50%.

It may be observed from Table II that the tumour dialysate was capable of hydrolysing up to 60 m*M* concentration of glutamine. On the other hand, the dialysate obtained from kidney produced maximum amount of ammonia at 20 m*M* substrate concentration.

The Figure indicates that the kidney dialysates, when incubated at varying pH levels from 5.0–8.8 in the presence of 5 m*M* glutamine, showed peaks at 2 different pH vs: pH 5.0 and 8.0. But the tumour dialysate had the maximum activity at pH 8.0. In this case, however, the activity increased with increase in pH, till it attained the maximum at pH 8.0.

Table I. Effects of dialysates obtained from kidney and tumour on glutamine degradation

Tissues	Homogenate after dialysis	Dialysate	Dialysate (boiled for 10 min)
Kidney	893 ± 60	14,60 ± 100	156 ± 9
Tumour	1294 ± 100	17,60 ± 95	9411 ± 10

All values are μg NH_4^+/100 mg protein.

Table II. Effects of dialysates on increasing concentrations of glutamine

Tissues	Glutamine concentration (m*M*) 5	10	20	40	60
Kidney	8,333 ± 100	5,238 ± 500	14,285 ± 1,560	13,095 ± 965	8,333 ± 250
Tumour	4,545 ± 410	7,575 ± 675	9,090 ± 828	25,000 ± 2,065	65,909 ± 5,100

Values indicated are μg NH_4^+/100 mg protein.

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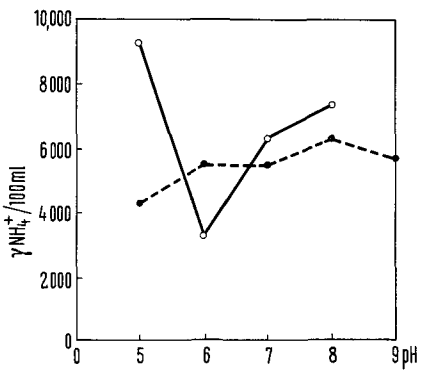
Discussion. The results presented here indicate that some factor which is also capable of hydrolysing glutamine could be dialysed out of the homogenates of both the normal kidney and the tumour. The presence of proteins in the dialysates and also the fact that this factor was heat-labile indicate the possibility of an enzymatic nature of this factor. This may be more clearly indicated by the high affinity of the dialysates towards the substrate and also by their pH optima.



Glutamine hydrolysing capacity of the dialysates obtained from kidney (normal) and tumour homogenates at different pH. ○—○, Kidney dialysate; ●—●, tumour dialysate.

The data presented here might indicate the possibility of the glutaminase being broken down into smaller molecules and the active core of the enzyme may be passing through the bag. Further work is, however, in progress to characterize this protein and study its properties.

Résumé. Dans les reins et dans les tissus de tumeur un facteur provoquant une glutaminase a été mis en évidance. Cette activité est plus faible dans les reins. Le dialysat du rein accuse deux pH optima de 5.0 à 8.0, tandis que celui de la tumeur n'a qu'un pH optimum de 8.0.

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Inhibition of Galvanic Skin Response by the Splanchnic Afferent

Preliminary experiments have shown that electrical stimulation of the somatic afferent nerve, as well as the splanchnic and the vagal afferent nerves, causes inhibition of galvanic skin response (GSR) in cats[1]. Very little is known, however, about the nature of the primary afferent concerned with these phenomena. In the present paper, the component of the splanchnic afferents participating in the reflex inhibition of GSR was investigated from the correlation between the neurogram and inhibitory effects of the splanchnic stimulation.

Material and methods. Under ether anesthesia, bronchial canula and saphenous vein canula were inserted into 16 cats. GSR was recorded from the paws of the anterior limbs by means of Ag-AgCl electrodes. EEG, femoral arterial pressure and heart rate were recorded simultaneously. The great splanchnic nerve was severed just proximal to the coeliac ganglion and was isolated from surrounding tissues up to the sympathetic trunk intrathoracically. The animals were immobilized with Flaxedil and maintained with positive pressure respiration. Stimulation electrodes were laid on the proximal portion of the nerve and the neurogram of the whole nerve was recorded at the distal end. All the nerves exposed were protected with warmed paraffin-oil. Rectangular pulses of 0.01 msec duration with variable intensities were applied repetitively, usually at a frequency of 5/sec.

In order to evoke GSR, the common peroneal nerve was stimulated every 45 sec for 200–500 msec with a train of pulses of 5 msec duration at a rate of 10/sec and an intensity adjusted to evoke GSR. In order to keep the amplitude of evoked GSR constant for a long period, body temperature was maintained constant and the depth of anesthesia (Urethane or Nembutal) was adjusted to a state in which evoked GSR was large and stable and spontaneous background activities were small.

Results and discussion. After securing constant amplitude of evoked GSR, splanchnic stimulation was applied for a few minutes, including 2 or 3 evoked GSR within the stimulation period. As shown in Figure A, evoked GSR was depressed almost completely during the period of splanchnic stimulation. In 30 out of 82 cases, depression of amplitude of GSR due to splanchnic stimulation was observed without changes in blood pressure; in 41 cases, it was accompanied by a fall of blood pressure and in 11 cases by a rise. Thus it can be concluded that the depression of GSR is not due to changes in the blood pressure. The neurogram of the splanchnics revealed 4 components, when increasing intensity of the stimuli. The first 3 components of the compound action potential of the splanchnic nerve are shown in Figure B. As was to be expected, threshold for the first component (TI) was lowest. Threshold of the second, the third and the fourth component was approximately 3, 8, and 25 times higher than TI. Relationship between intensity of stimulation and the inhibitory effect was examined in 9 cases, giving all similar results. Figure C shows one typical example. Mean amplitude of 2 GSR during splanchnic stimulation period and of 6 GSR in the control period (each 3 successive GSR of before and after splanchnic stimulation) were measured. In the ordinate, the degree of inhibition of GSR is expressed as

$$\left(1-\frac{\text{mean amplitude of GSR during stimulation period}}{\text{mean amplitude of GSR in the control period}}\right)\times 100.$$

The abscissa shows the intensity of the stimulus expressed in multiples of TI. Stimulation of the first component fibers only was not accompanied by any changes on GSR. Depression of GSR set in when stimulus intensity was elevated to excite the fibers of the second component, the

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