on frog's rectus muscle and effect of TTC on action of different agonists on other muscle preparations is established, an opportunity would be available to search for a relationship, if any, between the pharmacological effects of TTC and its biochemical actions[12–18]. However, it should be mentioned that structurally TTC possesses 1 quaternary nitrogen in the tetrazole ring, generally an essential requirement for acetylcholine antagonists. Future investigation would disclose how far other salts of tetrazolium behave differently to TTC[19].

Zusammenfassung. Es wurde die Wirkung eines Tetrazolsalzes (2:3:5, Triphenyl-Tetrazolium-Chlorid) auf die durch Acetylcholin hervorgerufene Kontraktion des M. rectus abdominis beim Frosch geprüft. In verhältnismässig niedriger Dosierung verhält sich die Substanz wie ein kompetitiver Hemmer des Acetylcholins, während sich dieser Effekt in höherer Dosierung als nichtkompetitiv erwies.

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Impairment of Growth and Pancreatic Hypertrophy in Rats Fed Trypsin Inhibitor from Raw Peanuts

Raw soybean meal retards growth in young animals causing, at the same time, pancreatic hypertrophy[1–3]. In chicks impairment of growth and pancreatic hypertrophy may result from feeding of the soybean trypsin inhibitor (SBTI) but may also occur after inhibitor-free meal[1,2]. In rats more consistent changes are produced by SBTI, while heat-inactivated preparations are harmless[3]. The mode of stimulatory action is still unknown. Feeding of raw soybean meal also prolongs blood coagulation in chicks[4], but it seems unlikely that the inhibitor, being a foreign protein, could be absorbed in amounts sufficient to influence coagulation.

Another trypsin inhibitor, extracted from raw peanuts (*Arachis hypogaea*), was found to decrease the spontaneous fibrinolytic activity of blood in man[5,6] and to enhance experimentally induced arterial disease in rabbits[7]. Raw peanut meal was reported to impair growth in young pigs[8]. It was, therefore, of interest to see if trypsin inhibitor fractions from raw peanuts, like SBTI, would retard growth and produce pancreatic hypertrophy in young rats.

The trypsin-inhibitor fraction was prepared from hexane-defatted raw peanut meal[9]. Assayed against crystalline trypsin (Novo Laboratories, Copenhagen) 1 mg of the peanut fractions neutralized about 0.07 mg trypsin. Inhibitor-free preparations were obtained from solutions kept for 2 h in a boiling waterbath before precipitation with acetone. The peanut inhibitor is a stronger inhibitor of activator-induced fibrinolysis than SBTI[10]. The preparations were fed to weanling male rats (25–30 g, Sprague-Dawley strain) in a dosage of 50 mg daily/animal mixed in Purina Chow. The drinking water contained 1.0 mg ascorbic acid/animal per day. Body weights were recorded weekly for each animal. After 6 weeks, the animals were killed with ether and the pancreas carefully isolated and its wet weight recorded. All other organs were inspected morphologically. Paraffin sections were prepared from the pancreas and appropriately stained (Haematoxylin-Eosin). The results of a comparative assay with 10 animals in each group are presented in the Table.

There was a significant retardation of growth and an increase in pancreas weight in the group fed the trypsin inhibitor fraction. In both cases the p-value was less than

Body weights and pancreas weights in weanling rats fed trypsin-inhibitor fractions or heat-inactivated preparations from raw peanuts

	Inhibitor material	Heat-treated material
Original body weight (g)	27.9 ± 1.8 25–30	28.1 ± 1.4 25–30
Final body weight (g)	94.7 ± 14.5 70–126	114.5 ± 11.5 94–130
Gain in body weight (g)	66.8 ± 14.7 56.3–95.0	86.4 ± 9.2 65.4–103.3
Pancreas weight (mg)	641 ± 56 530–760	421 ± 31 380–480
Ratio of pancreas to body weight	0.0068	0.0037

10 animals in each group. Results expressed as mean ± standard deviation and with range.

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0.01. Macroscopical examination of the other organs did not reveal any abnormalities similar to those of the pancreas. Studied histologically, the pancreas from the inhibitor group appeared more fragile though the overall architecture of the tissue was normal. There was increased vascularity. The acinar tissues were hyperplastic with increased basophilic staining of alveolar cells and with less zymogen granules in their cytoplasm. The number of alveolar cells per acinus was significantly increased over the control, but the appearance and distribution of inert cells did not visibly differ. These results compare well with those observed after raw soybean meal in rats.

Although the peanut preparations were impure, the inertness of the heat-treated material would suggest that the trypsin-inhibitor was the responsible agent. The decrease in zymogen granules would suggest that the pancreatic changes were caused by epithelial hyperplasia with increased vascularity and hyperemia. Presence of incompletely digested protein in the intestinal tract has been suggested as a cause of the pancreatic response. This raises the possibility of a yet unknown, feedback mechanism controlling pancreatic size and growth. However, in man protein malabsorption is not correlated with an increase in size of the pancreas[11].

Zusammenfassung. Trypsinhemmende Präparate aus rohen Erdnüssen (*Arachis hypogaea*) wurden während 6 Wochen jungen Ratten verfüttert. Das normale Wachstum wurde verzögert und eine Hypertrophie des Pankreas mit gleichzeitiger Hyperplasie der serösen Drüsenzellen bewirkt. Hitzebehandelte Präparate hingegen waren wirkungslos.

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Vasoactivity of Human Plasma and Plasma Protein Fractions

Application of plasma from dogs[1] and rabbits[2] to rabbit aortic strip preparations has been found to cause contractions which cannot be attributed to known vasoactive substances. Several workers[1,3,4] have also referred to a potentiation of responses of this preparation to a variety of substances by small concentrations of plasma and it has been suggested that this is due to the albumen content[4]. During the course of experiments using isolated perfused vessels in the apparatus described by DE LA LANDE and RAND[5], we have found that veins from the rabbit ear are sensitive to small doses of plasma kinins but relatively insensitive to other known vasoactive substances. Such preparations however respond to injections of small amounts of plasma. Further work has shown similar constrictor responses to human plasma in isolated perfused vessels from many sources. These include small subcutaneous and mesenteric veins from man, dog subcutaneous veins and both veins and arteries of the rabbit. However, the most consistently sensitive preparation in this regard is the central vein of the rabbit ear which we have used to investigate the nature of the substance or substances in plasma responsible for the constrictor activities. Injection of as little as 0.002 ml of human plasma into a 10-ml/min flow of Krebs solution through the lumen of the vein may initiate a constriction (Figure 1a). It is usually found that the sensitivity of a vessel to plasma initially increases progressively following successive injections of plasma, but then attains a relatively stable level (Figure 1b). Responses to all other vasoactive agents are also elevated by plasma pretreatment. These results have been interpreted as evidence that plasma has both intrinsic vasoactivity and also non-specific potentiating effect which is only slowly reversible. These actions may or may not be due to the same substance.

The central vein of the rabbit ear and also all other perfused vessels investigated, respond to certain Cohn fractions of human plasma proteins with constrictions similar to those caused by whole plasma. Fraction III-0 is the most potent and many ear veins respond to 0.1 mg or less. There is a close similarity between the dose-response curves for III-0 and plasma (Figure 2). Fresh plasma from 7 subjects was found to have activity equivalent to that of 2.2–4.6 mg/ml of III-0 (Table I). This activity was stable at room temperature for many hours but during storage of plasma at − 20 °C declined slowly relative to freshly prepared III-0 solutions. Constrictor activity in other Cohn fractions is less than that of III-0; fraction IV-1 has approximately 40% of the constrictor activity of III-0/mg dry material. The other fractions tested were III-1, IV-4, IV-5, IV-6, IV-7 and V and all showed less than 10% of the constrictor activity of III-0. Although fraction V, which is 98% albumen, has virtually no constrictor activity it reproduces the non-specific potentiating effect of plasma. Perfusion of albumen concentrations of 1 mg/ml produced detectable potentiation of the effects of noradrenaline in the rabbit ear artery and of bradykinin (BK), III-0 and IV-1 in the rabbit ear vein. There was no conspicuous evidence of potentiation with any Cohn fraction except V.

Constrictor activity in plasma and Cohn fractions can be distinguished from that due to catecholamines, histamine, 5HT, angiotensin, ATP, vasopressin, oxytocin and prostaglandin E_1 by the use of antagonists or by considering the nature of the rabbit ear vein preparation, which is relatively insensitive to most vasoactive substances except plasma kinins. There is, however, marked similarity between responses to bradykinin and the plasma venocon-

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