was absorbed out with washed human red cells[7]. A preparation was also fractionated on Sephadex G100 columns. The absorbed or fractionated extracts were titrated for haemagglutinating activity with washed human red cells, and mitogenic activity was assessed by adding samples to human lymphocytes and measuring the uptake of tritiated thymidine after 3 days in culture[8].

Electrophoretic mobility measurements were made on cell suspensions in TC 199. Lymphocytes were used at concentrations of 5×10^6 cells per ml and macrophages at 3×10^6 per ml. Cell suspensions were incubated with PHA at a final dilution of 1/600 or 1/3000 for 1 h at room temperature before measurements were made. Mobility was measured in a Zeiss Cytopherometer at 25 °C on cells not washed after incubation with PHA and controlled against untreated cells in TC 199.

Results showed that absorbtion reduced the titres of the haemagglutinin to 1:2 or zero from an initial 1:10,000 whilst approximately 70% of the mitogenic activity remained. Column fractionation produced 1 fraction with haemagglutinating activity alone, but failed to give any component containing mitogen alone. The Table gives the results of electrophoretic measurements and shows a marked reduction in mobility of lymphocytes after treatment with unabsorbed PHA, and with the haemagglutinating fraction. This reduction of mobility, however, is almost completely abolished when absorbed PHA is used.

Effect of phytohaemagglutinin preparations with and without haemagglutinating activity on electrophoretic mobility of a) lymph node cells and b) peritoneal macrophages

a) *Lymphocytes*[a]

Sample	Migration time (sec)	Change from control (%)	Viability (%)
Control	7.35	0	62
+ PHA 1/600 (native)	9.35	28	47
+ PHA 1/3000 (native)	8.60	17	42
+ PHA 1/600 (haemabsorbed)	7.10	−3	52

b) *Macrophages*[b]

Sample	Migration time (sec)	Change from control (%)
Control	6.70	0
PHA 1/600 (agglutinin, no mitogenic activity)	8.50	27
PHA 1/600 (haemabsorbed, 70% mitogenic activity)	6.85	4

[a] Mean of 2 separate experiments. [b] Mean of 4 separate experiments. Each single result represents the mean of 20 electrophoretic measurements.

The results suggest that the haemagglutinating fraction may be responsible for the electrophoretic change. These findings, whilst confirming the results of earlier experiments with PHA[5,9], showed that the mitogenic component of PHA does not necessarily react on the surface of the cell, at least not to the extent of bound molecules causing an alteration of surface charge. Fractionation of similar commercial preparations of PHA[10] yielded 3 different components 2 of which were mitogenic, whilst a more recent study yielded 2 glycoproteins[2,11] of high and low mitogenic activity with an inverse ratio of haemagglutinating activity. The starting material indeed showed no fewer than 17 components on acrylamide electrophoresis. This biochemical complexity was supported by marked variations in response of lymphocytes stimulated with different preparations of PHA in culture[12]. It is suggested that some agglutinating and mitogenic properties of PHA may occur either on the same molecule[2] or 2 molecules firmly bound to each other. This may explain the absorption of mitogen by lymphocytes[4] and also provide some explanation for the attractive, but possibly misleading, surface hypothesis.

Zusammenfassung. Die elektrophoretische Mobilität von Lymphozyten und Makrophagen wird durch Phytohaemagglutinin um mehr als 20% erniedrigt, wobei das letztere nach Absorbierung des erythrozytagglutinierenden Komponenten diese Eigenschaft verliert.

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Specifity of the Immunological Inhibition of the Parathyroid Hormone Activity

In the past few years a great progress has been noted in our knowledge about mode of action and the structure of the polypeptide chain of parathyroid hormone[1]. On the other hand, the immunological activity and specifity of this hormone has not been finally determined. The aim of this work was to find out if there is any correlation between the biological and immunological activity of the hormone and the problem of its specifity.

Materials and methods. The sources of parathyroid hormone came from fresh human (from autopsy), bovine or pig glands (from slaughter house). Immunization was performed using rabbits of the Vienna white race. The activity of the hormonal preparations was measured on parathyroidectomized Sprague-Dawley rats.

Two kinds of the active hormonal material were used: native gland homogenate, called in this work extract

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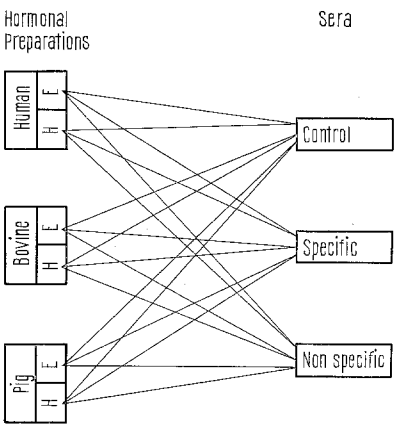
and purified hormonal material, prepared by phenol-extraction procedure[2], called in this work hormone. Immunization of rabbits by the gland antigens prepared according to ŚWIERCZYŃSKA[3] consisted of 2 i.m. injections of antigen weekly, for 3–4 weeks. The activity and specifity of sera were confirmed by haemagglutination technique according to Boyden and gel-precipitation reaction according to Ouchterlony. The hormonal activity was measured in calcium-mobilizing test[4] and by evaluation of the alkaline phosphatase activity[5]. The calcium and phosphorus in the urine were determined by CLARCK and COLLIP[6] and by FISKE and SUBAROWA[7] methods. The activity of alkaline phosphatase was measured in the blood by the KING and AMSTRONG method[8]. The plan of experiments is presented in the Figure. The extracts and hormones were incubated with the sera (specific, non-specific and control serum of rabbit) for 45 min at a temperature of 37 °C in relation of 0.2 ml of hormonal preparation to 2 ml of serum in dilution 1/16. The optimal dilution of sera was determined by gel-precipitation technique. After the incubation, the activity of the mixture was evaluated in the biological tests.



Plan of experiments. E, extract; H, hormone.

The second series of experiments were performed in vivo. The sera has been administrated i.p. to rats 30 min before the i.m. injection of hormonal material. The results obtained in the biological tests by administration of the hormonal preparation only were taken as 100%. The results obtained from hormones treated with the sera were expressed also in percent by comparison with this activity.

Results. The activity of purified hormonal material was always higher than that of the extracts. Simultaneously the quantity of protein in hormones was lower than that of the extracts. In the extracts it varied from 1.35 to 2.62 mg/ml, while in the hormone from 0.11 to 1.25 mg/ml.

The immunological inactivation of hormonal materials in vitro and in vivo represents significant inhibition of the hormone's and extract's biological activity (Tables I and II). This phenomenon could be observed only when the specific sera were used. All non-specific sera did not give inhibition of the hormonal activity. The degree of inhibition of the hormonal activity in calcium mobilizing test and alkaline phosphatase activity was always different. The specific sera inhibited especially the calcium-mobilizing effect, however the alkaline phosphatase activity was inhibited to a lower degree. The inhibition of calcium mobilization was very significant and very

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Table I. Inhibition of the activity of purified hormonal preparation (% of activity)

		Sera		
		Specific	Control	Non specific
Calcium-mobilizing test	in vitro	45.53 ± 13.2[a] (9)	93.47 ± 1.8 (9)	93.36 ± 18.6 (9)
	in vivo	39.63 ± 5.3[a] (9)	100.2 ± 6.2 (9)	93.19 ± 10.4 (9)
Alkaline phosphatase activity	in vitro	63.05 ± 17.8[a] (9)	95.68 ± 9.1 (9)	89.00 ± 19.7 (9)
	in vivo	76.18 ± 22.6[a] (9)	91.21 ± 6.2 (9)	90.36 ± 23.2 (9)

[a] Difference statistically significant. Number of experiments in parentheses.

Table II. Inhibition of the activity of parathyroid gland homogenate (% of activity)

		Sera		
		Specific	Control	Non specific
Calcium-mobilizing test	in vitro	25.98 ± 9.6[a] (9)	98.73 ± 10.2 (9)	109.91 ± 15.5 (9)
	in vivo	35.96 ± 12.3[a] (9)	111.29 ± 14.1 (9)	110.62 ± 18.2 (9)
Alkaline phosphatase activity	in vitro	96.14 ± 19.2 (9)	103.66 ± 9.1 (9)	99.69 ± 22.1 (9)
	in vivo	87.32 ± 19.8 (9)	100.06 ± 14.7 (9)	124.76 ± 23.7 (9)

[a] Difference statistically significant. Number of experiments in parentheses.

similar results were obtained from in vivo and in vitro experiments. It is also characteristic that full inhibition of the hormonal activity was never obtained, independently of the dilution of specific sera and the time of inactivation. There always remained some residual hormonal activity.

Discussion. These results indicate that the different biological activity of the parathyroid hormone may be connected with the different active centers of hormone. Moreover the immunological activity is connected to a high degree with a fragment of hormone which is probably the same as this one responsible for the calcium mobilization phenomenon[1].

Parathyroid hormone has a great immunological specifity. It was not possible to obtain any form of the immunological reaction with non-specific sera. In all experiments the biological inactivation of hormonal materials was obtained only when the specific antisera were used. It is not in agreement with the former results of KOOH and FRASER[9], which observed the inhibition of the guinea-pig, rabbit and rat parathyroid hormone activity after administration of anti-bovine PTH serum.

The influence of the parathyroid hormone inactivation on the phosphorus release was also investigated but no significant differences were observed. It is in agreement with the opinion that phosphorus metabolism is independent from the parathyroid glands, but rather depends on the dietary content of the mineral[10,11].

Résumé. Des préparations hormonales de parathyroïde humaine, bovine et porcine furent traitées par des sérums anti PTH spécifiques et non spécifiques. Les expériences in vitro et in vivo ont montré que l'inhibition de l'activité biologique du PTH se produit seulement sous l'influence des sérums spécifiques. L'inactivation immunologique n'inhibe jamais complètement l'activité du PTH.

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X-Irradiation and Thyroid Activity in a Teleost, *Mystus vittatus* (Bloch)

Some investigators have reported similarity of dose-survival time curves after X-irradiation between mammals and few teleosts[1-4]. Extensive studies on the thyroid physiology of mammals in response to ionizing radiation, and very little work in this field on teleostean thyroid, present an uneven picture of the thyroid activity in this group. Therefore, in this experiment an attempt has been made to study the effects of varying doses of X-irradiation on thyroid gland activity in a freshwater catfish, *Mystus vittatus*. This species was specially selected for the present program because some aspects of its thyroid activity under natural and varied experimental conditions are well known[5-10]. Thyroidal radioiodine (^{131}I) uptake and its histology were taken as parameters for the measurement of thyroid activity, which were done by the procedure described earlier[6].

210 adult males of *M. vittatus* were utilized in this experiment. 180 specimens were divided into 6 batches of 30 each. Their pharyngeal area containing thyroid follicles were exposed to varying doses of X-rays and their thyroid activity at regular intervals of 1, 2, 3, and 4 months were studied (Table). The X-ray apparatus was operated at 250 kvp, 30 ma, with 0.5 mm Al filter. The temperature for experimental and control groups was kept more or less uniform and it ranged from 22 to 24 °C. Exposure of pharyngeal region to the dose of 0.8 kR (Batch 1) initially accelerated the thyroidal radioiodine uptake. Specimens of batch 2 showed slight reduction in thyroid activity

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Thyroid activity of *Mystus vittatus* in response to varying doses of X-rays

Batch[a]	Dose in kR/fish	Maximum of ^{131}I uptake after irradiation at various intervals (%)			
		1 month (mean ± S.E.[b])	2 months (mean ± S.E.)	3 months (mean ± S.E.)	4 months (mean ± S.E.)
1	0.8	16.00 ± 1.49 (8)	19.23 ± 2.00 (7)	20.15 ± 1.55 (6)	19.77 ± 1.20 (6)
2	1.6	15.40 ± 0.86 (6)	13.32 ± 0.54 (7)	11.80 ± 0.77 (6)	10.00 ± 1.15 (6)
3	2.4	13.50 ± 1.20 (6)	10.24 ± 1.10 (6)	9.79 ± 0.40 (7)	7.50 ± 0.48 (6)
4	3.2	9.37 ± 0.76 (5)	5.64 ± 0.53 (6)	4.58 ± 0.53 (5)	3.00 ± 0.22 (5)
5	4.0	6.29 ± 0.49 (5)	4.00 ± 0.28 (5)	2.17 ± 0.54 (5)	2.18 ± 0.44 (6)
6	4.8	4.13 ± 0.32 (5)	2.37 ± 0.18 (5)	1.70 ± 0.23 (5)	
7	Sham-irradiated control	14.56 ± 1.22 (8)	13.67 ± 0.88 (6)	15.00 ± 1.39 (6)	16.24 ± 0.76 (6)

[a] Each batch had 30 specimens. [b] Mean with S.E. Number of fishes used for the evaluation of thyroid activity at various intervals are given in parentheses.