loss is statistically significant only in methanol. The authors believe this loss to be connected with the elution of lipids and some other N and P containing substances whose exact chemical nature, however, has not been verified in the present work. Nitrogen loss was slight, reaching its highest value – 5% – in methanol. The phosphorus loss was much more important, attaining in methanol as much as 40%. N and P loss is not associated with the elution of nucleic acids and proteins; it probably involves micromolecular compounds, phospholipids and phosphoproteids. Minimal P and N loss and no loss of dry mass were observed when acetone was used. The latter to be the best substituting medium, all the more so, since it is also a good fixative used in enzyme histochemistry[18-19]. Methanol and ethanol, though used in various laboratories, seem to be less valuable. Higher alcohols (butanol and propanol) are highly viscous at low temperatures and thus require a prolonged substitution time.

Conclusions. (1) Owing to the elution of tissue substances in the course of substitution, the percentual values of dry mass were lower as those of the controls. This loss was statistically significant only in the case when methanol was used. (2) The percentual loss of nitrogen caused by elution by the substituting medium is in general not large; most marked in methanol and ethanol and smallest in acetone. (3) The percentual loss of phosphorus extracted by the substituting media differs widely from one medium to another reaching about 40% in methanol and 20% in ethanol, while in acetone no loss is observed. (4) On the basis of the results obtained, the authors reach the conclusion that acetone is the most suitable medium as far as extractability of N and P, and dry mass loss are concerned.

Résumé. Les auteurs ont examiné les possibilités de diminution du contenu en azote et phosphore et de la masse sèche au cours de la congelation-dissolution des tissus. La diminution la plus marquée a été observée après l'usage de méthanol. Tandis qu'avec l'acétone elle est insignifiante.

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Department of Histology, School of Medicine, Warsaw (Poland), October 16, 1961.

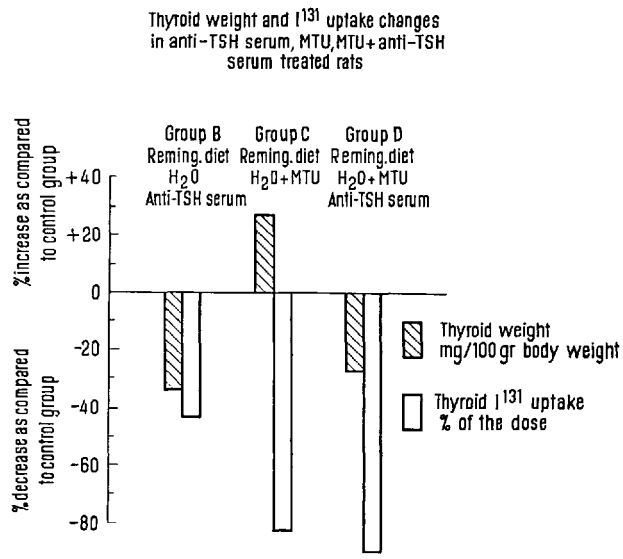
[18] G. BERG and L. G. BARTH, Anat. Rec. *117*, 520 (1953).
[19] A. G. E. PEARSE, *Histochemistry, Theoretical and Applied* (J. & A. Churchill Ltd. London 1960).

Inhibition of Endogenous TSH in Rats by Antiserum to Bovine TSH

The antigenic activity of pituitary gland hormones has been known for a long time, and since 1934 COLLIP et al. had extracted a serum inhibitory to the thyrotropic hormone. It is a well known fact that both in animals and humans exogenous TSH activity gradually diminishes down to nothing after a treatment lasting longer than 15 days. The refractoriness to TSH is not produced, or it appears later on, in various experimental situations, such as pregnancy, associated treatment with ACTH (BASCHIERI and FERRI[1]), or following the use of special preparations of TSH, such as those containing flavianic acid (WERNER[2]). More recently, further studies on anti-TSH serum have clarified the immunological character of sera obtained with bovine TSH (WERNER et al.[3,4]). It has also been found that such sera can inhibit, in the mouse, thyrotropic activity of exogenous TSH (CLINE et al.[5]). The purpose of the present study has been to ascertain whether anti-TSH-serum can inhibit endogenous TSH in an animal species (rat) different from the one (beef) whose TSH had been used to produce anti-TSH serum in a third species (rabbit). The effects of anti-TSH serum was evaluated in rats fed Remington's diet (low iodine diet) and in rats on the same diet but also treated with methylthiouracil (MTU).

Methods. 2 female rabbits (body weight about 2 kg) were treated with TSH emulsion and Freund's complete adjuvant. The antigenic mixture was administered subcutaneously, and the injections given at 7 days intervals for 4 weeks. Each weekly injection contained 4 I.U. of TSH (Ambinon-Organon) and 1 ml of Freund's adjuvant; 5 days after the last subcutaneous injection, the animals were given 2 I.U. of TSH in saline solution intravenously.

Therefore, at the end of the treatment, each rabbit had received 18 I.U. of antigen. 10 days after the end of the treatment, a blood sample from the animals was tested by the agglutination technique, and the immune serum was found to have a 1:540 titer. The biological activity of this serum was afterwards tested in rats. 4 groups of rats, 5 male animals in each group (Wister, 300 g average body weight) were studied for this purpose. The first group (A), already fed Remington's diet + distilled water for 10 days, were given one intramuscular injection of 0.1 ml of normal rabbit serum every second day. The second group (B), on the same diet + distilled water, were given one intramuscular



[1] L. BASCHIERI and F. FERRI, Folia endocrin. *4*, 359 (1951).
[2] S. C. WERNER, Endocrinology *22*, 291 (1938).
[3] S. C. WERNER, B. C. SEEGAL, E. OTERO-RUIZ, and R. W. BATES, Nature *185*, 472 (1960).
[4] S. C. WERNER, B. C. SEEGAL, and E. F. OSSERMANN, J. clin. Invest. *40*, 92 (1961).
[5] M. J. CLINE, H. A. SOLENKOW, and M. S. BROOKE, Endocrinology *67*, 273 (1960).

injection of 0.1 ml of anti-TSH serum every second day. The third group (C) on the same diet, was given a free amount of a suspension with 0.3 mg/ml of MTU orally; this group received 0.1 ml of normal rabbit serum intramuscularly every second day. The fourth group (D), under the same conditions as group C, received one intramuscular injection of 0.1 ml of anti-TSH serum every second day.

On the 12th day, each rat was given 8 μc of carrier-free radioactive iodine. After 24 h, all rats were killed with ether, and their thyroid glands were immediately removed and weighed. Thyroid radioactivity was then evaluated as % of the dose formerly administered, comparing it with a standard specifically prepared. Afterwards, the thyroid compounds were studied according to the chromatographic technique of TONG and CHAIKOFF[6].

In the present study, we have only examined the MIT/DIT ratio, which becomes rapidly altered during goiter-producing situations (iodine deficiency, treatment with MTU) (QUERIDO et al.[7]; PITT-RIVERS et al.[8]).

Standard deviation of results was evaluated by following formula:

$$\sigma m \sqrt{\frac{S \times^2}{(n-1).n}}$$

Parameter t was calculated as follows:

$$t = \frac{M_1 - M_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad \text{Where } \sigma = \sqrt{\frac{Sx_1^2 + Sx_2^2}{(n_1-1)+(n_2-1)}}$$

Results (summarized in the Table) show: (a) Thyroid weight in animals fed Remington's diet and treated with anti-TSH serum (group B) is markedly lower (34.7 %) than in control group (A). (b) Thyroid uptake of I^{131} under the same experimental conditions is distinctly impaired (43 %) by anti-TSH serum. (c) MIT/DIT ratio does not seem to be affected following treatment with anti-TSH serum. (d) As well known, thyroid weight in animals treated with MTU and normal rabbit serum (group C) is higher (28.6 %) than in control group (A). Under the same experimental conditions, thyroid uptake is strongly reduced (82 %). (e) Thyroid weight is much lower (47 %) in animals treated with MTU and anti-TSH serum (group D) than in animals treated with MTU only (group C) or than in those of the control group (A); it is practically the same as the thyroid weight in animals fed Remington's diet and treated with anti-TSH serum (group B). (f) Thyroid uptake in rats treated with MTU and anti-TSH serum (group D) is the lowest we found, as it is also 52 % lower than the one found in group C. (g) MIT/DIT ratio appeared definitely to be higher following MTU administration (3.7 as compared with 0.9 in control group). Anti-TSH serum simultaneous administration brought this relation back to values observed in group A and B.

Discussion. Our study confirmed that it is possible to obtain in rabbits an anti-TSH serum by using bovine hormone as antigen. Such serum can inhibit not only exogenous TSH, as observed by CLINE et al.[5], but also endogenous TSH in rats. The findings of WERNER et al.[3,4] *in vitro*, concerning cross reactions with bovine anti-TSH serum and pituitary hormones in mice and other animals, were thus confirmed *in vivo*.

Anti-TSH serum has affected both thyroid weight and radio-iodine uptake but the latter has shown more evident changes. These changes were observed both in animals fed low iodine diet and in animals in which MTU administration had prompted a stronger response to TSH. This proves that the serum which we prepared had high biological anti-TSH activity.

The specific anti-TSH activity is proved by the changes produced in groups C and D, in which groups we observed that this serum not only reduced the hyperplasia induced by MTU administration, but also brought the weight of the thyroid down to figures lower than in the control group (A). Thyroid uptake in group D is further reduced, as compared with its reduction due to MTU treatment. In group D along with the metabolic block from MTU administration, the thyrotropic stimulation, which still allowed some radio-iodine uptake in the thyroid gland. has also been inhibited.

In the present study, we have also dealt, in a preliminary way, with the problem of anti-TSH serum activity on thyroid hormonogenesis; we observed that such serum maintained MIT/DIT ratio in normal range, cancelling the

[6] W. TONG and I. L. CHAIKOFF, J. biol. Chem. *232*, 939 (1958).
[7] A. QUERIDO, K. SCHMIDT, and J. TERPSATRA, Ciba Foundation Coll. Endocr. *10*, 124 (1957).
[8] R. PITT-RIVERS, D. HUBBLE, and S. H. HOUTHER, J. clin. Endocr. Metab. *17*, 1313 (1957).

Group and number of animals	Treatment	Body weight g	Thyroid weight mg % of body weight	Thyroid I^{131} uptake % of the dose	MIT/DIT Ratio
A(5)	Remington diet, H_2O, Normal serum	304 ± 10	11.5 ± 1.57	10.7 ± 0.82	0.9
B(5)	Remington diet, H_2O, Anti-TSH-serum	309 ± 10.6	7.5 ± 0.56	6.1 ± 0.56	0.9
C(5)	Remington diet, H_2O + MTU, Normal serum	295 ± 17	16.1 ± 1.92	1.9 ± 0.1	3.7
D(5)	Remington diet H_2O + MTU, Anti-TSH serum	309 ± 16	8.4 ± 0.27	0.9 ± 0.08	0.8
t parameter					
A – B		—	3.6	8.75	
A – C		—	2.7	15	
C – D		—	5.6	10	
B – D		—	0.8	13	

Theoric t ($P < 0.05$) = 2.3; ($P < 0.01$) = 3.35; ($P < 0.001$) = 5.04.

deep changes induced by MTU treatment. The significance of these last findings deserve further attention and study.

Riassunto. Gli autori hanno ottenuto in conigli un siero anti-TSH usando come antigene ormone bovino. Questo siero inibisce il TSH endogeno di ratti nutriti con dieta di Remington e trattati con tiouracile, impedendo così la formazione della iperplasia tiroidea. È stato anche osservato che il trattamento con siero anti-TSH mantiene normale il rapporto MIT/DIT cancellando le salienti alterazioni indotte dal trattamento con tiouracile.

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On the Controlling Function of the Kidneys in Fibrinolysis

The lytic action of urine upon impure fibrin clots[1,2] was elucidated when a plasminogen activator, named urokinase (UK) was found in urine[3–5].

It is supposed that UK is a plasma plasminogen activator which, after it has been transferred from the tissues to the blood and carried to the kidneys, is excreted with the urine[6,7].

ASTRUP and STERNDORFF[3] suggested that UK may be a product of the kidney, a suggestion supported by other observations[8]. It can therefore be accepted that the fibrinolysis activator reaches the blood from the kidney tissue as it does from the other tissues.

So far no investigations, apart from the evaluation of the excretion of UK with the urine, have been carried out on the possibility of the secretion of this product by the kidney into the blood. It may be expected that, if UK is secreted by the kidney into the blood, the fibrinolytic activity of the renal venous blood will be higher than that of renal arteries.

In the investigations presented in this communication, the fibrinolytic activity of euglobulins from the renal arterial blood (RAB) or blood euglobulins from other vessels is compared with the fibrinolytic activity of euglobulins from the renal venous blood (RVB).

Methods. The human blood was collected from surgical patients during operations on the kidneys from renal vessels and cubital veins.

In rabbits under ethyl ether anaesthesia, the abdominal cavity was opened and the blood was collected from the renal vessels and other blood vessels.

Renal blood stasis was induced by pressure on the renal vein near to the vena cava.

Urine stasis was induced by pressure on the region of the ureter nearest to the kidney.

Blood was withdrawn into 0.1 *M* potassium oxalate (9/1:v/v). The euglobulins were obtained after diluting the oxalated blood plasma 10 times and precipitating at pH 5.3 by the addition of *N*/6 acetic acid. The precipitate was dissolved in borate buffer (pH 7.6) to half of the original plasma volume.

Clotting was induced after the addition of an equal volume of thrombin[9] (bovine thrombin 4 units/ml, containing 0.0125 *M* $CaCl_2$).

The fibrinolysis time was estimated at a temperature of 37°C from the moment when calcified thrombin was added.

Results. The difference in the fibrinolysis time of RAB and RVB euglobulins in rabbit is presented in Table I.

The fibrinolysis time of the euglobulins from RVB is shorter than that from RAB. Renal blood stasis causes a further decrease of fibrinolysis time (Table II).

Ligation of the ureter itself without pressure on renal veins causes such an increased activation of fibrinolysis

Tab. I. Fibrinolysis of the euglobulin fraction of rabbit's blood.

Number of cases	Euglobulin plasma fraction derived from		Fibrinolysis time in min
15	Arterial blood		198 (98–280)
	Renal venous blood		
15	Before stasis:		103 (18–172)
15		5 min	86 (10–155)
12	After stasis:	10 min	38 (0–60)
12		15 min	18 (0–47)

Tab. II. Fibrinolysis of the euglobulin fraction of rabbit's blood before and after stasis of the urine

Euglobulin fraction; Blood derived from		Fibrinolysis time in min Rabbits I	II	III	IV	V	VI
Femoral vein		164	128	110	273	190	–
Renal artery		182	119	92	280	175	170
Renal vein							
Before stasis of the urine		75	65	66	170	141	120
After stasis of the urine	10 min	45	14	–	120	95	20
	20 min	14	18	–	–	42	22
	30 min	no clot appeared			28	–	32

Tab. III. Fibrinolysis of human euglobulin plasma fraction in min

Cubital veins	Renal arteries	Renal veins
155	130	18
136	—	17
150	142	35
192	—	34
193	187	72

[1] W. SAHLI, Pflügers Arch. ges. Physiol. *36*, 209 (1885).
[2] R. G. MACFARLANE and J. PILLING, Nature *159*, 779 (1947).
[3] T. ASTRUP and I. STERNDORFF, Proc. Soc. exp. Biol. Med. *81*, 675 (1952).
[4] J. R. WILLIAMS, Brit. J. exp. Path. *32*, 530 (1951).
[5] G. W. SOBEL, S. R. MOHLER, N. W. JONES, A. B. C. DOWDY, and M. M. GUEST, Amer. J. Physiol. *171*, 768 (1952).
[6] K. N. VON KAULLA and H. SWANN, J. Thoracic Surg. *36*, 519 (1958).
[7] F. E. SMYRNIOTIS, A. P. FLETCHER, N. ALKJAERSIG, and S. SHERRY, Thromb. Diath. haem. *3*, 257 (1959).
[8] K. N. VON KAULLA and N. RIGGENBACH, Thromb. Diath. haem. *5*, 162 (1960).
[9] H. KOWARZYK, M. RECHNIC, M. SIWIŃSKA, and B. CZERWIŃSKA, Arch. Immunol. i Terapii Doświ. *6*, 475 (1958).