végétalisés. En présence de chloramphénicol seul (0,5 mg/ml), des pluteus se développent. Dans les cultures contenant du chlorure de lithium seul (0,0065 *M*) les pluteus présentent quelques traces de végétalisation indiquées par l'étroitesse du lobe oral et de la bouche et les parois épaissies de l'archentéron. En contraste les embryons traités simultanément par le chloramphénicol et le chlorure de lithium ne se développent jamais en pluteus; ils forment des larves fortement végétalisées constituées d'une petite vésicule ectodermique à parois minces et d'une énorme vésicule entodermique à parois épaisses.

Nous avons également examiné les effets du chloramphénicol sur l'animalisation. En raison de ses propriétés végétalisantes, on peut en effet prévoir que le chloramphénicol sera capable d'atténuer ou même de supprimer complètement les effets de l'animalisation. Nous avons effectivement observé que le chloramphénicol utilisé à des concentrations de 0,8 à 0,5 mg/ml inhibe les effets animalisants des ions zinc. En présence d'ions zinc (1×10^{-4} *M*), on obtient des blastulas animalisées. Leur touffe ciliée apicale est hyperdéveloppée et son extension correspond sensiblement aux types $^1/_2$ et $^1/_4$ selon la classification de HÖRSTADIUS[10]. Dans les cultures contenant du chloramphénicol (0,8 mg/ml) et du chlorure de zinc (1×10^{-4} *M*) aucune blastula animalisée ne se développe. L'une de ces cultures a donné 37% de petits pluteus, 40% d'embryons prismatiques, 14% de gastrulas chez lesquelles l'archentéron est seulement ébauché et enfin 9% d'embryons présentant une petite évagination de l'archentéron. Le chloramphénicol inhibe également l'animalisation provoquée par le bleu d'Evans. L'inhibition de l'animalisation provoquée par le zinc ou le bleu d'Evans est d'autant plus forte que la concentration en chloramphénicol est plus élevée.

Ces expériences montrent que le chloramphénicol exerce des effets végétalisants typiques, comparables à ceux déjà obtenus avec le lithium et avec la phénazone[11]. Les effets du chloramphénicol et ceux de la phénazone présentent en outre un certain nombre de particularités communes intéressantes. Ces deux agents ont en effet une activité antimitotique importante[12,13]. La formation de blastulas permanentes dans les cultures continues s'observe également avec le chloramphénicol et avec la phénazone. Nous avons observé au cours de ces recherches que les ions potassium n'empêchent pas les effets végétalisants du chloramphénicol ni ceux de la phénazone. A cet égard les effets de ces deux agents diffèrent de ceux du lithium dont les effets végétalisants sont supprimés en présence de concentrations suffisantes d'ions potassium[14,15]. Les analogies observées entre les effets exercés sur le développement de l'œuf d'oursin par le chloramphénicol et par la phénazone peuvent se montrer utiles pour l'interprétation des effets pharmacodynamiques de la phénazone. Chez les bactéries où ses effets ont été particulièrement étudiés, le chloramphénicol inhibe la synthèse des protéines par un processus d'ailleurs non élucidé. En outre, aux concentrations où il inhibe la synthèse des protéines, cet agent provoque l'accumulation d'une forme particulière et instable d'acide ribonucléique[16]. De nouvelles recherches sont nécessaires afin d'établir si de telles perturbations du métabolisme des protéines et des acides nucléiques s'observent également chez les œufs d'oursin traités par le chloramphénicol. Le fait que le chloramphénicol exerce des effets végétalisants comparables à ceux du lithium suggère que le lithium agit sur la détermination embryonnaire en perturbant le métabolisme des protéines et des acides nucléiques. Une analyse comparée des effets du chloramphénicol et du lithium sur le métabolisme des protéines et des acides nucléiques permettra de préciser ce point de vue. Outre le chloramphénicol, d'autres agents sont également capables d'interférer avec la synthèse des protéines et des acides nucléiques. Dans un prochain travail nous examinerons les effets de quelques-uns de ces agents et les comparerons avec ceux du lithium et du chloramphénicol.

Summary. Chloramphenicol, an inhibitor of protein synthesis, causes a strong vegetalization of whole larvae of sea urchin *Paracentrotus lividus*. Combination of chloramphenicol and lithium causes a stronger vegetalization than either of these agents alone. Chloramphenicol suppresses the animalizing effects of Evans blue and zinc ions. The effects of chloramphenicol are compared with those of lithium and phenazone. It is suggested that vegetalizing effects of chloramphenicol are connected with its inhibitory action on protein synthesis and the perturbations of ribonucleic acid metabolism.

R. LALLIER

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Quantitative Investigation of the P and N Loss in the Rat Liver when Using Various Media in the 'Freeze-Substitution' Technique

It has been established in the preceding paper[1] that the solubility of proteins in tissues fixed by the freeze-substitution technique is comparable to that in control and lyophilized material. This solubility as may be surmised is an indication of certain losses in tissue substances in the course of histological procedure. The determination of these losses is of great importance in the developing quantitative histochemical methods. Many authors [2–10] have discussed this problem in relation to various histochemical fixatives. The F-S method enables the application of various media and temperatures[11–14] and this may lead to various degrees of extraction of the tissue substances.

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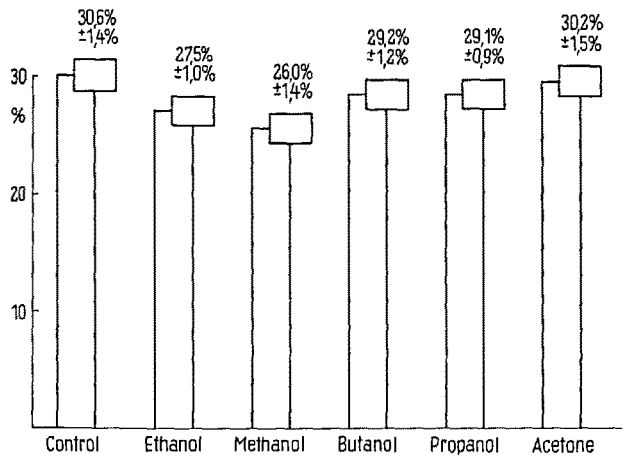

Fig. 1 represents percentual changes of the dry mass of the substituted liver in different media.

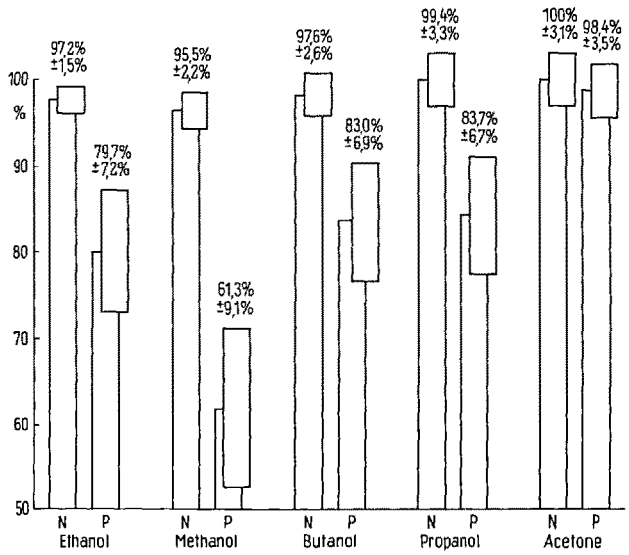

Fig. 2 represents percentual changes of N and P contents of the substituted liver in different media.

In the present work, nitrogen and phosphorus loss, as well as the decrement of rat liver dry mass during substitution in most commonly used organic solvents, was studied.

Material and Methods. For the experiments, livers of nine female rats of the Wistar strain, weighing about 150 g each, were used. Samples weighing 30-100 mg were weighed out on strips of aluminium foil quenched in isopentane at −105°C. After this treatment, the samples were placed in 5 ml of substituting medium at −79°C. From each liver, 5 samples were prepared and substituted in absolute ethanol, methanol, butanol, propanol and acetone. Further three samples served as controls. The material was substituted for 6–9 days at the temperature of the cooling mixture of dry ice and ethanol. The end of substitution was established by observing the cross section of control fragment of substituted tissue in butanol—the most difficultly penetrating alcohol. After substitution, the material was dried at 100°C to constant weight in order to determine the percentage of dry mass and compare it with the control. The individual samples were placed in Kjeldahl flasks and ignited in a selenium mixture[15] for simultaneous N and P determination. The quantity of dissolved phosphorus was also determined in the substituting media. Nitrogen was determined by the Parnas-Wagner modification of the Kjeldahl method[16], the methodical error under the given conditions being 1.7%. Phosphorus was determined by the Fiske-Subbarow method modified by HORECKER[17]. The error in phosphorus determination was 2.5%. The necessary statistical calculations were made.

Results. The average values of the results obtained are shown in the Table and illustrated by Figure 1 and 2. The sum of phosphorus found in the tissues and in the substituting media amounted always to about 100% of total P as compared with control tissues.

Discussion. The data given in the Table and Figures show that the percentage of the changes in the dry mass caused by elution of the substances by the media used are only slight; most marked in methyl and ethyl alcohol. The

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The content of N, P, and dry mass of rat liver after the substitution in the different organic solvents. Results expressed as % values of the control material.

	Control			Ethanol			Methanol		
	% of dry mass	mgN/ 100 mg[a]	µgP/ 100 mg[a]	% of dry mass	% of content N	P	% of dry mass	% of content N	P
Mean	30.6	3.55	514.2	27.5	97.2	79.7	26.0	95.5	61.3
Standard deviation	± 1.4	± 0.37	± 60.0	± 1.0	± 1.5	± 7.2	± 1.4	± 2.2	± 9.1
Variation coefficient	4.6	10.4	11.7	3.6	1.5	9.0	5.4	2.3	14.8

	Butanol			Propanol			Acetone		
	% of dry mass	% of content N	P	% of dry mass	% of content N	P	% of dry mass	% of content N	P
Mean	29.2	97.6	83.0	29.1	99.4	83.7	30.2	100.0	98.4
Standard deviation	± 1.2	± 2.6	± 6.9	± 0.9	± 3.3	± 6.7	± 1.5	± 3.1	± 3.5
Variation coefficient	4.1	2.7	8.3	3.1	3.3	8.0	5.0	3.1	3.6

[a] of the fresh weight

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.

K. OSTROWSKI, J. KOMENDER, H. KOŚCIANEK, and K. KWARECKI

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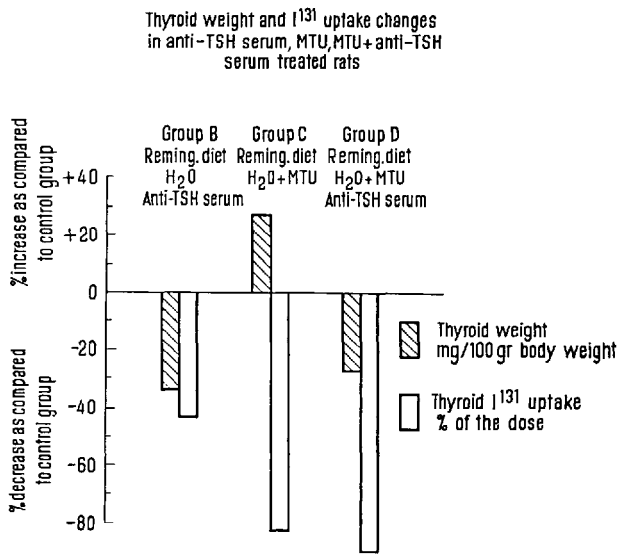
Inhibition of Endogenous TSH in Rats by Antiserum to Bovine TSH

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



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