

with arginine-vasotocin, and this solution was injected into 5 mice, as previously described (group C).

Results. The Table summarizes the results of our experiments with synthetic arginine vasotocin, performed with immature mice as test animals.

Discussion. The Table shows that arginine vasotocin, whether incubated with half-anterior hypophyses for 3 h 30 min or mixed with the incubation solution of these organs from male rats incubated alone, can diminish significantly the influence of that incubation solution on the uterus weight when injected into immature mice.

As REVIERS and MAULÉON⁶ have shown, the bioassay of IGARASHI and McCANN⁷, which is based on the uterus weight of immature mice, is not very specific for measuring the quantity of follicle stimulating hormone (FSH) in a solution. Therefore the influence of a solution on the uterus weight has to be interpreted cautiously. We studied the weight of the uterus and the weight of the ovaries and completed our bioassay by a histological examination of the ovaries.

Our results show further that only in group B1 does the weight of the ovaries differ significantly from the weight of the ovaries of the animals in group A. In group B1 we have also found that the maximum diameter of the follicles is a little smaller than that of the animals of group A. However, the maximum diameter of the follicles of the ovaries of group B₁ is much larger than that of the animals sensitized with 0.25 IU of HCG. Previously we have shown that fresh rat pineal bodies⁴, acetone-dried sheep pineal powder⁴ and a fraction F3 of a sheep pineal extract after gel-filtration on Sephadex G-25 (Fine)⁵ can diminish or inhibit the secretion of the anterior hypophysis into the incubation medium, so that the ovaries of the test animals show a growth of the follicles comparable with that of the ovaries of the mice sensitized with HCG only. On the contrary, the ovaries of the mice injected with the incubation solution of anterior hypophysis alone show a cer-

tain growth of the follicles. The effects observed in group B1, group B2 and group C are comparable. Therefore we believe arginine vasotocin to act on the gonads or on the gonadotrophic hormone(s) and not on the secretion of the anterior hypophysis in vitro, as we have observed in in vitro experiments with acetone-dried sheep pineal powder^{4,8,9}.

Résumé. L'arginine vasotocine est capable d'inhiber la réponse de la souris impubère à la stimulation gonadotrope, mais ne semble pas agir directement in vitro sur l'excrétion hypophysaire, comme le fait le facteur inhibiteur épiphysaire présent dans la poudre totale et dans la fraction F3, obtenu après filtration d'un extrait épiphysaire sur gel Séphadex G-25 (Fine).

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⁷ M. M. DE REVIERS and P. MAULÉON, *C. r. hebd. Séanc. Acad. Sci., Paris* 267, 540 (1965).

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The Cortisol Metabolism of Human Pulmonary Tumour Tissue

Malignant cells are known to metabolize cortisol. Cells originating from lymphosarcoma are able to oxidize or reduce at C-11 and C-20¹. BURTON² reported on similar results in his mouse and rat experiments. We too were able to demonstrate steroid metabolism in different transplantable rat tumour tissues³. HALEY⁴ investigated the corticoid metabolism of cancer tissue in mammary cancer and observed different steroid metabolisms of individually different efficiencies without establishing a morphological correlation. To the best of our knowledge the steroid hormone catabolic activity in human malignant pulmonary tumour has not yet been studied.

Material and methods. Sections of a surgically removed tumour were kept on ice and processed within 1 h. 1 g of the apparently intact tissue was cut up and the slices were placed into 30 ml of Krebs-Ringer bicarbonate solution. 1 μ C 1-2 ³H cortisol in 0.1 ml of alcohol solution was added (1 mC/mg cortisol), and was incubated at 37°C under a continuous stream of CO₂ + O₂ gas mixture. 1 g of the same patient's pulmonary tissue was incubated as a control. The medium was extracted with chloroform (3 \times vol) evaporated in vacuo. The extract was first purified⁵ and the steroids separated in a Bush B5 solution system. To ensure identification, in parallel test acetylation was per-

formed for 24 h with pyridine and acetaldehyde, and the material was run beside cortisol acetate and cortisone acetate standards in a Bush 3 system. The activities of the paper strips (cut into 1 cm segments) were measured with the 'Packard Tricarb' liquid scintillation spectrometer. Histological investigation was carried out on every tumour.

Results and discussion. In the Table the cases are arranged according to the intensity of observed metabolism. This is shown in the third column. In the first 3 cases no metabolism was observed; transformation ratio increased rising from the fourth to the thirteenth case. With No. 14 a considerable quantity of polar fraction was obtained. A transformation product of this kind

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⁴ H. B. HALEY, D. F. DIMICK and M. B. WILLIAMSON, *Surgery Gynec. Obstet.* 123, 812 (1966).

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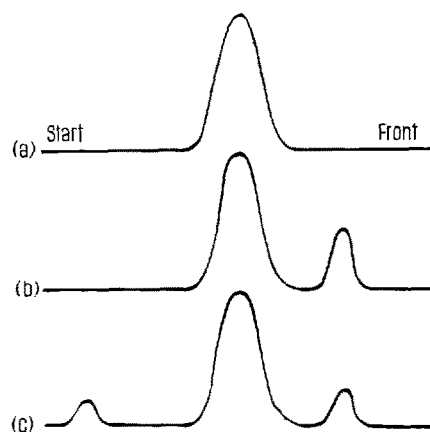
Comparison of cortisol metabolism and the histological types of 14 pulmonary tumour tissue

No.	Total recovery (% of starting radiation activity)	Chromatographic separation (% of cortisol)			Necrosis	Histological types of lung cancer
		Cortisone	Uncategorized catabolites Polar	Apolar		
1	30	—	—	—	extensive	Squamous cell carcinoma
2	50	—	—	—	extensive	Squamous cell carcinoma
3	52	—	—	—	extensive	Squamous cell carcinoma
4	67	6.0	—	—	extensive	Squamous cell carcinoma
5	88	7.5	—	17.5	extensive	Well-formed columnar cells, a greater degree of polymorphysm
6	73.5	7.8	—	—	minimal	Squamous cell carcinoma, a greater degree of polymorphysm
7	74.0	7.9	—	—	minimal	Squamous cell carcinoma
8	89	8.3	—	—	minimal	Adenocarcinoma, well-formed cubical and columnar cells
9	67	8.8	—	3.2	extensive	Glandular structure, differentiated columnar cells
10	75.0	9.3	—	16.0	minimal	Undifferentiated small cell carcinoma
11	80.0	15.8	—	7.1	—	Poorly differentiated squamous cell carcinoma
12	70.0	30.0	—	—	minimal	Undifferentiated squamous cell carcinoma
13	99.7	50.1	—	—	—	Undifferentiated 'oat-cell' carcinoma
14	67.5	8.1	8.2	—	minimal	Bronchoadenoma with mucous secretion

was not found in the other 13 cases. In pulmonary tumours a non-polar fraction must also be taken into consideration; this we were able to demonstrate in 4 cases (column 5). In the last column of the Table the morphological character of each pulmonary tumour is shown. If we compare the differentiation of tumours with the transformation ratio of cortisol into cortisone, we find that the less differentiated the lung tumours the greater are their steroid metabolization abilities. The control pulmonary tissue showed no such transformation under these experimental conditions. The Figure illustrates the idealized radiochromatogram of 3 histologically different pulmonary tumours, a squamous cell carcinoma, and undifferentiated 'oat-cell' carcinoma and a bronchoadenoma.

We believe that the considerable cortisol-cortisone transformation observed in non-differentiated pulmonary tumours deserves attention. The phenomenon is caused by the increased activity of the 11- β -hydroxy-dehydrogenase enzyme. DOUGHERTY pointed out a similar correlation for lymphoid cells. He definitely maintained that the less mature a lymphoid cell the higher is its 11- β -hydroxy-dehydrogenase activity. In the tumours with differentiated tissue, structure transformation was of a lower degree. Though as far as we were able to judge with the naked eye, our studies were carried out on tissue sections without necrosis. The recovery of radioactivity was the least in the first three cases. Therefore the possibility has to be considered that the steroid binding ability of the necrotic

tissue might be different from that of the intact tissue. We observed a similar phenomenon in our earlier experiments on transplantable rat tumours. One of the interesting properties of the pulmonary tumours is their production of an ACTH-like substance. This fact was demonstrated



Idealized representation of chromatogram shows separation of cortisol and its metabolites in Bush B5 solvent system of 3 pulmonary tumours: (a) Squamous cell carcinoma of lung, (b) undifferentiated 'oat-cell' carcinoma of lung, (c) bronchoadenoma.

first by LIDDLE^{6,7} and was confirmed by other authors⁸. Our results direct attention also from another aspect, namely that of the significant steroid metabolism in pulmonary tumour tissue to the endocrine involvement of pulmonary tumours. Under in vitro conditions only the renal tissue (apart from the liver) is able to perform a transformation of such extent.

According to JENKINS⁹ data, all other organs examined possess this ability only to a very slight degree¹⁰.

Zusammenfassung. Bösartige Lungengeschwulstgewebe verschiedener Zelltypen wurden mit Hydrocortison $1\text{ }^3\text{H}$,

$2\text{ }^3\text{H}$ inkubiert. Die freien, markierten Steroide wurden extrahiert, chromatographisch getrennt und identifiziert.

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Peroxidase Isoenzyme Associated with the *Aegilops umbellulata* Chromosome Segment Transferred to Chinese Spring (*Triticum aestivum*)

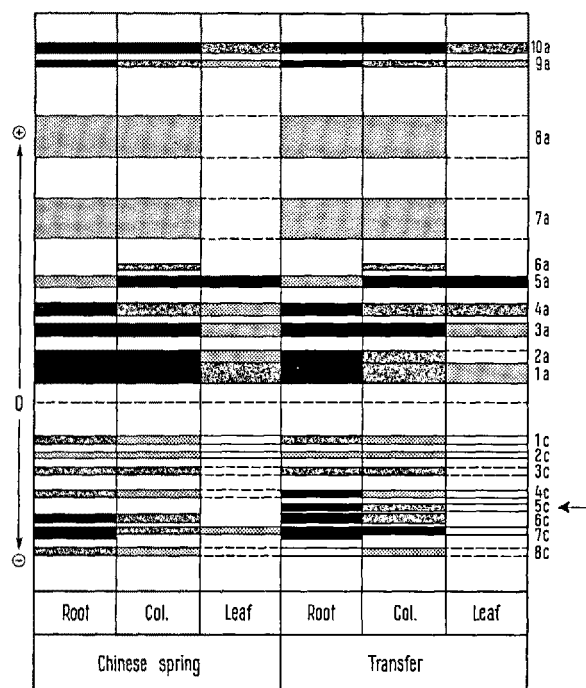
The resistance to leaf rust (*Puccinia recondita* Rob. tritici) transferred from *Aegilops umbellulata* as a translocation into spring bread wheat (*Triticum aestivum*), by SEARS¹ was shown by SOLIMAN et al.² to be a simply inherited character. SEARS³ had also shown that this translocation was on chromosome 6B and that the translocated chromosome segment was small and terminal.

By the use of polyacrylamide gel electrophoresis techniques, BHATIA and SMITH⁴ had found that the leaf protein extracts from 12-day-old seedlings of Transfer (IC 13296) had 2 additional protein bands as compared to the parent variety, Chinese Spring. They had interpreted the additional protein bands to be causally related to the genetic information carried by the introgressed *umbellulata* chromosome segment into Chinese Spring. Studies were therefore undertaken using horizontal gel electrophoretic techniques to study the peroxidase isoenzyme differences in different tissues of seedlings of Chinese Spring and Transfer (IC 13296).

The seeds were germinated in petri dishes on wet filter paper in dark at 20°C for 48 h and later grown under continuous illumination in a control environment. The seedlings were harvested after 8 days. The leaf, coleoptile, and the roots were separated for each of the seedlings, and similar tissues bulked together from all the seedlings in a sample. The tissue samples were either used as fresh or were frozen at -10°C for later use. 1 g of tissue was ground with 100 mg of acid washed sand and 0.1 ml of a freshly prepared mixture of 2 parts of 12.5% glucose in 0.02M Trizma base [Tris (hydroxymethyl) amino methane] solution (adjusted to pH 7.5 with HCl), and one part of aqueous solution of 0.8% NaCl + 0.2% NaNO₃. The general electrophoretic procedures of BREWBAKER et al.⁵ were followed for isoenzyme separation and staining in polyacrylamide gels. The gels were stained for peroxidase employing O-dianisidine as the hydrogen donor.

Peroxidase isoenzymes were detected on both anodal as well as cathodal sides of the gel. The zymogram showing the peroxidase isoenzymes is diagrammatically presented in the Figure.

The isoenzymes on the anodal side of the gel showed variation among the 3 tissues with regard to the intensities



Diagrammatic representation of the peroxidase isoenzymes in the extracts from the root, coleoptile and leaf of 8-day-old seedlings of 'Chinese Spring' and Transfer (CI 13296) carrying *umbellulata* chromosome segment. The additional band in the Transfer tissues is marked with an arrow.

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