Résumé. On a ajouté des antigènes aux cultures in vitro des cellules mononucléaires obtenues du péritoine des 3 groupes de cobayes, le premier étant normal, le deuxième présentant une sensibilité immédiate et le troisième une hypersensibilité retardée à la tuberculine ou à l'ovalbumine. L'addition de l'antigène spécifique aux cultures des cellules obtenues des cobayes avec hypersensibilité retardée a provoqué la formation de cellules avec 2 ou plusieurs noyaux. Ceci suggère une relation étroite entre l'hypersensibilité retardée et la formation de cellules multinucléaires.

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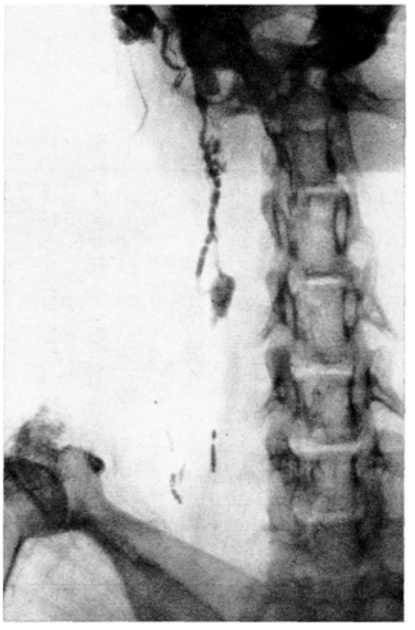
Prolongation of Skin Homograft Survival by Local Intralymphatic Radioisotope Injections

The importance of regional lymphatic tissue in antibody formation was first demonstrated by McMASTER and HUDACK[1] and confirmed by EHRICH and HARRIS[2]. It was also shown that selective reduction of lymphocytes is a means of homograft survival[3]. Attempts were therefore made to achieve a prolongation of homograft survival by selective reduction of lymphocytes with the intralymphatic irradiation of the lymphoid tissues[4,5]. These procedures involved an introduction of large doses of radioisotopes. In the present study an attempt was made to produce a prolongation of homograft survival by selective irradiation of regional lymph nodes with small doses.

In the rabbit, lymphatics draining from the ear before their confluence with the cervical veins pass through only 2 agglomerations of lymphoid tissue: the retroauricular and the cervical. These lymph nodes can be injected from the central lymphatic channel of the ear. Six rabbits therefore received an intralymphatic injection of 1 mC ^{111}Ag colloid (particle size 10–100 nm) into their right ear, 7 animals were injected with ^{131}I-Lipiodol ultra fluid (radioactive dose 0.5 mC). The volume of injected fluid was 0.3–0.5 ml. Twenty-four h after the injection a skin homograft was made on the injected right ear. The skin flap removed from the right side was transplanted onto the non-irradiated left ear of the same animals (technical control). The dimensions of the grafts were 15 × 15 mm. In 6 controls the grafting procedures were duplicated without isotope injection.

The homografts were rejected in the controls on the 7th to 9th day (mean 7.8 ± 0.4 days), in the ^{111}Ag-injected animals on the 11th to 14th (mean 12.5 ± 0.7) day, and in the ^{131}I-Lipiodol-injected on the 12th to 16th (mean 13.9 ± 0.5) day. Moreover, in this series, in 3 out of 7 animals the rejection was incomplete and the reaction was limited to only a small part of the transplant.

The distribution of the injected radioisotope was studied by the injection of 3–4 μC ^{131}I-Lipiodol or ^{111}Ag-colloid diluted to 0.4–0.5 ml with non-radioactive Lipiodol or colloid solution. The animals were killed 1 h after the intralymphatic injection. The regional lymph nodes, the spleen, liver and lungs were removed and digested with 20% sodium hydroxide and the radioactivity measured



Retroauricular and cervical lymph nodes of a rabbit injected with ^{131}I-lipiodol ultra fluid.

[1] PH. D. McMASTER and S. HUDACK, J. exp. Med. *61*, 783 (1935).
[2] W. E. EHRICH and T. N. HARRIS, J. exp. Med. *76*, 335 (1942).
[3] D. D. McGREGOR and J. L. GOWANS, J. exp. Med. *117*, 303 (1963).
[4] J. R. WHEELER, W. F. WHITE and R. Y. CALNE, Br. med. J. *2*, 339 (1965).
[5] C. CHIBA, M. KONDO, M. ROSENBLATT and P. L. WOLF, Transplantation *5*, 232 (1967).

Tissue recoveries after intralymphatic injections of ^{111}Ag-colloid and ^{131}I-Lipiodol in the rabbit, expressed as % of injected dose.

	Lymph nodes		Liver	Spleen	Lungs	Injection site	Total
	Auricular	Cervical					
^{111}Ag	28.4 ± 5.9	23.0 ± 6.2	14.0 ± 0.5	0.01 — 1.7	0.02 — 0.1	8.6 — 18.7	69.4 ± 5.3
^{131}I	4.6 ± 0.97	2.8 ± 1.2	–	–	24.3 ± 2.2	17.1 ± 6.0	45.6 ± 8.3

in a scintillation-well detector. In the 8 ^{111}Ag-colloid-injected animals, over 50% of the injected dose was recovered in the regional lymph nodes; the particles leaving the lymphatics were mainly retained by the reticulo endothelial system and were recovered from the liver. In X-ray films, in most animals injected with ^{131}I-Lipiodol, an excellent filling of the regional lymphatics could be observed. It was, however, established that on the average less than 10% of the injected radioactive oil remains in the regional lymph nodes, about $^1/_4$ of the injected dose was recovered from the lung tissue. Total recoveries in the organs which were examined varied between 17 and 88% (mean 46%). In 5 dogs injected with 0.5 ml/kg ^{131}I-labelled triolein in the leg lymphatics 81% of the injected radioisotope was recovered from the lungs 4 h later[6].

Accordingly, it can be assumed that only a fraction of the injected radioactive oil remains in the lymphatic system; and it seems improbable that the intralymphatic fraction could be substantially increased by the reduction of the injected volume. Irradiation of regional lymph nodes by intralymphatic injections of radioactive substances is nevertheless an efficient means for the prolongation of homograft survival.

Zusammenfassung. Injektion von ^{131}I-Lipiodol oder ^{111}Ag-Kolloid beim Kaninchen in ein Ohrlymphgefäss verzögert die Abstossung eines Hautlappen-Hormotransplantes am Ohre wesentlich. Bei der Untersuchung der Verteilung intralymphatisch verabreichter Radioisotopen in den einzelnen Geweben wurde festgestellt, dass etwa 50% des ^{111}Ag-Kolloids, aber unter 10% des ^{131}I-Lipiodols von den regionären Lymphknoten aufgenommen wird und dass Lipiodol hauptsächlich im Lungengewebe, Ag-Kolloid in der Leber gespeichert wird.

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[6] G. Szabó, in *Progress in Lymphology* (Ed. A. Rüttimann; G. Thieme, Stuttgart 1967).

Excretion of C_{19}-Steroids in Human Seminal Fluid

Recently the presence of dehydroepiandrosterone (DHEA) glucuronoside[1] and sulphate[2] in human seminal fluid has been demonstrated. Since the direct conversion of circulating DHEA sulphate or sulphatide respectively apparently plays a major role in the metabolism of C_{19}-steroids[3–5], it seemed of interest to ascertain such processes in tissue connected with the formation of seminal fluid. The present communication therefore deals with the assay of free and conjugated C_{19}-steroids in this material following the administration of double-labelled DHEA sulphate.

Six hours after i.v. injection of 43.0 μg Na-7α-^{3}H-DHEA-^{35}S-sulphate with 4,640,000 dpm ^{3}H and 1,611,000 dpm ^{35}S (^{3}H/^{35}S = 2.88) into a 43-year-old male subject 4.3 ml of seminal fluid were obtained. The sample was diluted with water to 10 ml and thoroughly extracted with 3 × 20 ml chloroform for removal of free steroids. Subsequently, the preextracted material was treated with 60 ml ethanol-acetone (1:1 v/v). After filtration of precipitated proteins the filtrate was evaporated to approximately 2.5 ml, diluted with 12.5 ml chloroform-methanol (1:9 v/v) and submitted to ion exchange chromatography on activated and pretreated DEAH-Sephadex A-50[6]. Separation and purification of steroid sulphatides, sulphates and glucuronosides could be achieved by means of thin-layer chromatography on silica gel G in chloroform-methanol-ammonia (20:5:0.2 v/v). For cleavage of the conjugates the solvolysis in ether/perchloric acid proved efficient[7]. Free steroids were separated by thin-layer chromatography on silica gel G in chloroform-dioxane (94:6 v/v) (A), on aluminium oxide in cyclohexane-butyl acetate-butanol (50:45:5 v/v) (B), eventually subjected to the 2,4-dinitrophenylhydrazine reaction[8] and rechromatographed in system A, whereas the non-ketonic C_{19}-steroids were rerun in cyclohexane-ethyl acetate (1:2 v/v) (C). Aliquots of all fractions, localized in a Berthold Dünnschicht-Scanner LB 2720, were assayed for ^{35}S and/or ^{3}H in a Packard Tricarb Spectrometer 3003.

Of the 1520 dpm ^{3}H or 0.033% of administered ^{3}H-activity, found in the seminal fluid, free steroids represented 481 dpm ^{3}H (0.010%), sulphoconjugates 967 dpm ^{3}H (0.021%) and glucuronosides 72 dpm ^{3}H (0.002%). The fraction of sulphoconjugates consisted of steroid

^{3}H-labelled C_{19}-steroids in human seminal fluid after i.v. administration of double-labelled DHEA sulphate

Steroid	dpm ^{3}H in		
	free steroids	sulpho-conjugates	glucurono-sides
Dehydroepiandrosterone	158	440	23
Androsterone	128	214	22
Androstenedione	37	64	
Androstenediol	15	84	
Etiocholanolone	34	36	
Testosterone	40	19	
Androstenetriol		16	

[1] W. Dirscherl and H. Breuer, Acta endocr., Copenh. *44*, 403 (1963).
[2] O. Steeno, C. Schirren, W. Heyns and P. de Moor, J. clin. Endocr. Metab. *26*, 353 (1966).
[3] G. W. Oertel, P. Knapstein and L. Treiber, Hoppe-Seyler's Z. physiol. Chem. *345*, 221 (1966).
[4] E. E. Baulieu, C. Corpechot, F. Dray, R. Emiliozzi, M. C. Lebeau, P. Mauvais-Jarvis and P. Robel, Recent Prog. Horm. Res. *20*, 411 (1965).
[5] P. Knapstein, F. Wendlberger and G. W. Oertel, Experientia *23*, 480 (1967).
[6] G. W. Oertel, Hoppe-Seyler's Z. physiol. Chem. *336*, 236 (1964).
[7] L. Treiber and G. W. Oertel, Clinica chim. Acta *17*, 81 (1967).
[8] L. Treiber and G. W. Oertel, Z. klin. Chem. *5*, 83 (1967).