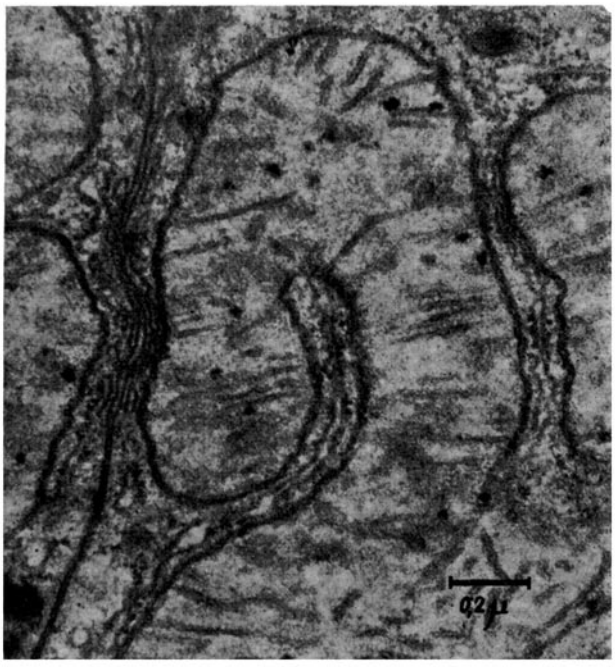
de «polyester» avec catalyseur et accélérateur, qui, enfermées dans une boîte contenant du desséchant, sont maintenues à 4° pendant 24 h, à 20° pendant 4 h et à 60° pendant 24 h.



Foie de souris fixé OsO_4 2% pH 7,2, inclus dans le «polyester». Grossissement 50000 ×.

Le polymère est plus dur et plus cassant que le méthacrylate. Il se coupe cependant très facilement à 200 Å avec des couteaux de verre cassés sous un angle d'environ 30°. Des angles plus grands ainsi que des couteaux métalliques aiguisés selon la méthode de SJÖSTRAND[2] fournissent des coupes plissées et gondolées.

Nous avons employé un microtome Trüb-Täuber modifié[4].

L'enrobage au «polyester» a été appliqué avec succès pour des fragments de foie (figure), pour des bactéries[5], des gamètes d'Allomyces, de jeunes racines et feuilles de diverses plantes[6]. Les bactéries et les gamètes ont été inclus dans de l'agar 1,5% immédiatement après la fixation pour faciliter les manipulations ultérieures[7]. Le même procédé, appliqué aux feuilles, permet d'orienter ces dernières dans la position voulue et empêche presque totalement leur détachement du reste du matériel d'inclusion.

La polymérisation du «polyester» employé est extrêmement régulière car sur une trentaine de séries d'inclusions nous n'avons jamais observé ni bulles ni gonflement. Des inclusions comparatives de bactéries dans du méthacrylate et dans du «polyester» ont montré de nombreuses fois que les bactéries avaient éclaté dans le méthacrylate mais étaient restées intactes dans le «polyester».

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Zusammenfassung

Zur Herstellung von Dünnschnitten für das Elektronenmikroskop wird eine neue Einbettungsmethode für biologisches Material beschrieben. An Stelle von Methacrylat wird ein Polyester verwendet, wodurch Unregelmässigkeiten in der Polymerisation vermieden werden können. Sukzessive Serieneinbettungen und Vergleichsstudien können mit grösserer Sicherheit ausgeführt werden.

The Incorporation of Carbon Labelled Precursors into the Nucleic Acids of Various Tissues in the Intact Rabbit

We have previously described the pattern of incorporation of ^{32}P into the nucleic acids of rabbit tissues *in vivo*[1]. Since most previous studies on the incorporation of carbon labelled precursors into the nucleic acids of the intact animal have been restricted to only a few tissues (for reviews see BROWN[2] and SMELLIE[3]), we have now examined the pattern of incorporation of carbon labelled formate, adenine and glycine into a series of rabbit tissues. For reasons of expense, the experiments were limited to a small number of animals and to a single incorporation time (2 h).

Female albino rabbits (about 1500 g) received 1 mc of the labelled precursor (^{14}C-formate, Na salt, or (2-^{14}C)-glycine or (8-^{14}C)-adenine sulphate) by intramuscular injection and the tissues were worked up to yield deoxyribonucleic acid (DNA), cytoplasmic ribonucleic acid (cRNA) and nuclear RNA (nRNA) as described by SMELLIE *et al.*[1]. The constituent bases after hydrolysis with 12 *N* $HClO_4$ at 100° for 1 h (WYATT[4]) were separated by chromatography, using isopropanol-HCl[5] as descending solvent and butanol ammonia[6] as ascending solvent. The bases were eluted and quantitatively estimated as described by CROSBIE *et al.*[7] and the radioactivity determined.

Wide variations were found in different animals but Table I shows some typical results for the purines and thymine. Incorporation into other pyrimidines was negligible. The pattern for DNA follows that found with ^{32}P, specific activities being high in such tissues as appendix and bone marrow in which cellular proliferation

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Table I. — Specific activities of nucleic acid bases (in counts/min/μm) in DNA, nRNA and cRNA in various rabbit tissues 2 h after administration of carbon labelled precursors

Tissue	Base	^{14}C-formate			($8-^{14}$C)-adenine			($2-^{14}$C)-glycine		
		DNA	nRNA	cRNA	DNA	nRNA	cRNA	DNA	nRNA	cRNA
Appendix	Adenine	3849	2650	1326	10385	19600	13160	180	520	122
	Guanine	510	913	822	462	1258	618	(0)	179	(0)
	Thymine	1104	—	—	—	—	—	2	—	—
Bone Marrow	Adenine	3810	2204	1986	14781	21700	10348	207	267	(900)
	Guanine	1560	1286	1136	2371	2600	422	(0)	606	(0)
	Thymine	3815	—	—	—	—	—	26	—	—
Intestinal mucosa	Adenine	317	1356	(27)	4737	17700	12400	(0)	112	(0)
	Guanine	316	3460	1197	1566	3470	410	(0)	142	(0)
	Thymine	270	—	—	—	—	—	20	—	—
Kidney	Adenine	31	163	202	1	14932	4760	(0)	69	(0)
	Guanine	0	584	136	161	1411	(0)	(0)	(0)	(0)
	Thymine	33	—	—	—	—	—	17	—	—
Liver	Adenine	31	86	23	1009	16506	1177	16	87	(0)
	Guanine	163	168	43	208	642	22	(0)	(0)	(0)
	Thymine	45	—	—	—	—	—	—	—	—
Spleen	Adenine	167	728	231	5800	18700	1	24	100	—
	Guanine	154	412	122	2480	2750	1020	31	224	—
	Thymine	156	—	—	—	—	—	—	—	—
Thymus	Adenine	353	656	[1]	4720	9831	5080	63	262	13
	Guanine	478	544	[1]	252	787	125	(0)	254	15
	Thymine	1	—	—	—	—	—	(0)	—	—

[1] Specimens not available. – Figures in parenthesis are derived from counts too low to be significant.

is active and low in kidney and liver in which mitotic activity is low. The specific activities of the purines in nRNA are high and in most tissues exceed the corresponding values for DNA and cRNA purines except in appendix and bone marrow in which the DNA purines give particularly high values. Incorporation of ($8-^{14}$C)-adenine occurs readily into nucleic acid adenine (and to a much lesser extent into the guanine) especially in lymphatic tissues and bone marrow; the specific activities of the nRNAs are high and the difference between nRNA and cRNA is particularly pronounced in liver and kidney. Incorporation of ($2-^{14}$C)-glycine is much less extensive but the differences between tissues are similar to those found with the other precursors.

Previous results with ^{32}P were presented graphically[1] for various times and the figures found for a 2 h incorporation period are summarized in Table II which shows the same general pattern with ^{32}P as with ^{14}C-labelled precursors. In general the difference between nRNA and cRNA is more pronounced with ^{32}P than with carbon labelled precursors and is especially pronounced in liver and kidney.

Table II. — Relative specific activities of DNA, nRNA, and cRNA 2 h after administration of radioactive inorganic phosphate to rabbits (data derived from the curves of SMELLIE *et al.*[1]).

	DNA	nRNA	cRNA
Appendix	1373	3765	852
Bone marrow	1289	3837	861
Intestinal mucosa	258	959	243
Kidney	81	769	138
Liver	61	599	50
Spleen	154	2236	292
Thymus	434	2229	310

BENDICH, RUSSELL, and BROWN[8] found the greatest uptake of ^{14}C-formate in the DNA of rat intestine and spleen and the least in brain, kidney, liver and testis, while TOTTER and his colleagues[9] have obtained results with formate incorporation into rabbit bone marrow with which ours are essentially in agreement. The incorporation of formate into DNA thymine which is of the same order as its incorporation into the DNA purines in nearly all tissues, is due presumably to the activity of the methyl group, which arises from C_2 intermediates[10]. *In vitro*, incorporation of formate is much more pronounced into thymine than into purines in bone marrow and Ehrlich ascites cells[11].

Comparison of the relative values for the DNA adenines in different tissues with respect to the utilization of different precursors shows a fair degree of agreement in the relative utilizations of the different precursors for DNA formation in accordance with the findings of PAYNE *et al.*[12] for the relative uptakes of carbon labelled formate, glycine, adenine and ^{32}P by mouse nucleic acids. Similar comparisons with RNA, however, show that the relative utilization of the different precursors in RNA formation is very different and that in several tissues adenine seems to be more readily incorporated into nRNA and cRNA than are formate or glycine.

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It has been suggested[13] that the biochemical stability of DNA in non-dividing cells is manifest only when ^{32}P and ^{14}C-adenine are used as precursors but in our experience the renewal patterns of DNAs from different tissues are quite similar regardless of the precursor used. The recent results of SIBATANI[14] who with the aid of an improved technique, finds negligible incorporation of ^{14}C-formate into the DNA of resting rat liver *in vivo* and *in vitro* are in agreement with our view.

Preliminary measurements on the labelling of the acid soluble adenine compounds at 2 h show that with ^{14}C-formate and ^{14}C-glycine the highest activities are observed in those tissues in which DNA purines are most active, liver and kidney giving much lower values. This effect may be partly due to differences in purine synthesis and partly to variations in pool sizes in different tissues. The low values found for the activities of nucleic acid purines after ^{14}C-glycine administration are almost certainly due to a very considerable dilution of the administered glycine since the acid soluble nucleotides in these experiments also showed very low activities. The observed differences between the acid-soluble adenine nucleotides in different tissues after ^{14}C-adenine administration are considerably smaller than with formate or glycine.

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Résumé

Les résultats expérimentaux montrent le degré d'incorporation du formate-^{14}C, de l'adénine-8-^{14}C et de la glycine-2-^{14}C dans l'ADN et dans les ARN nucléaire et cytoplasmique de l'appendice, de la mœlle osseuse, de la muqueuse intestinale, du rein, du foie, de la rate et du thymus du lapin *in vivo*.

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Reserpine and Human Platelet 5-Hydroxytryptamine

A decline in 5-hydroxytryptamine (5-HT) after the injection of reserpine into rats[1] and rabbits[2] and after repeated administration in man[3] has been reported. The observations described below show that in man a single dose will remove virtually the whole of the 5-HT in the platelets and that this is associated with an impaired ability of the platelets to absorb 5-HT.

The effect of 1 mg reserpine (i.m.) in man on the 5-HT content of the platelets and on their ability to absorb 5-HT

	ng 5-HT/10^8 platelets	ng 5-HT/10^8 platelets after incubation with 5-HT at 37° C for 2 h
Before reserpine. . .	36	173
1 day after reserpine	14	39
3 days after reserpine	< 1	36
9 days after reserpine	7	40

The methods used were those described by HARDISTY and STACEY[4] and a typical result is recorded in the table. It will be seen that 24 h after the intramuscular injection of 1 mg reserpine the amount of 5-HT in the platelets had fallen to 40% of its initial level and that after 3 days none could be detected. Incubation of 5-HT with platelet-rich plasma obtained before administration of the drug led to a large uptake by the platelets[4] but 24 h after reserpine this ability to absorb 5-HT was markedly reduced and impaired absorption persisted during the following days. By the 9th day the 5-HT content of the platelets showed evidence of recovery; in other subjects recovery started at about the same time and was still incomplete 3 weeks after the injection.

The prolonged reduction of 5-HT following reserpine has been advanced by SHORE *et al.*[2] as evidence that 'serotonin is formed in platelets at the time of their formation'. Bone marrow has little capacity for 5-HT synthesis and it would seem more likely, as postulated by TOH[6], that circulating platelets absorb 5-HT from organs where it is formed and that reserpine both liberates 5-HT from platelets and prevents further absorption.

Platelet life has been variously estimated from 5 to 9 days[7]. The present finding that at least 3 weeks is required for recovery after a single dose of reserpine suggests, since reserpine is rapidly eliminated[8], either that platelet life is longer than would appear from these estimates or that reserpine affects not only platelets in circulation but also the platelet bearing tissue, thus leading to the subsequent discharge of platelets with an impaired ability to absorb 5-HT.

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Zusammenfassung

Nach einmaliger intramuskulärer Injektion von 1 mg Reserpin sinkt beim Menschen der 5-Oxytryptamingehalt der Blutplättchen zwei Tage lang ab, um bis zum neunten Tag auf unbestimmbarem Wert zu verharren.

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