to the corresponding controls and to the corresponding unchromatographed extracts.

The results obtained from the assay of the eluates from chromatographic strips show that there is a big difference between the hearts of control and of treated animals, in contrast to the results obtained from the assay of the alumina extracts. α-M-NA was detected in the hearts of treated guinea-pigs as early as 1 h after α-M-DOPA administration. After 16 h, the pressor activity of the α-M-NA strip accounted for more than 70% of the pressor activity of the whole chromatogram. This activity declined slowly: after 6 days it was as high as 30%. In all instances the corresponding eluates of control hearts had the same activity as isotonic NaCl.

On the other hand, marked activities were found only in the NA strips for control hearts and only in the NA and α-M-NA strips for treated hearts. The assay of the eluates from chromatographic strips shows, therefore, besides the presence of α-M-NA, a profound and long lasting (> 6 days) depletion of NA in the hearts of treated guinea-pigs. The biological assay procedure is thus in agreement with the fluorimetric method. In view of this, it can be expected that a similar process occurs also in rat and mouse hearts. In fact, α-M-NA has been detected in the hearts of mice treated with α-M-DOPA[2].

It is interesting to note that after α-M-DOPA administration the missing NA is replaced in the heart muscle by an equipressor amount of α-M-NA. Moreover, under the experimental conditions used here, the vasoconstrictor effect of α-M-NA was as potent (on a molar basis) as that of NA, and this was true in animals having different sensitivities to NA (Figure 3).

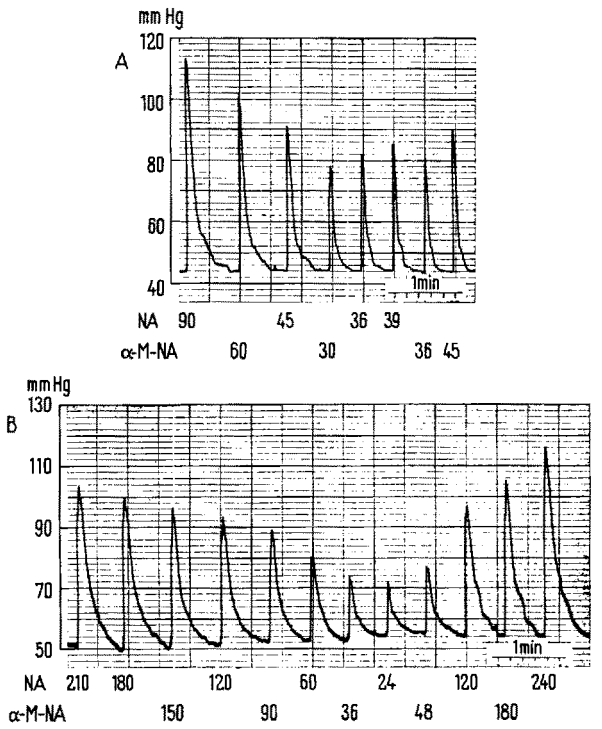

Fig. 3. Pithed rat preparations; arterial blood pressure. Vasopressor activity of NA, as compared with that of α-M-NA; doses are expressed in 10^{-12} *M*. Atropine (2 mg/100 g, s.c.) was given 10–15 min before operation. A: Rat ♂ 190 g. B: Rat ♂ 220 g.

This latter result does not agree with the observation made recently by DAY and RAND[14] who found that α-M-NA had less vasoconstrictor activity than NA on the blood pressure of the rat. In the light of the results reported here it would seem that the hypotensive action of α-M-DOPA does not consist in a depression of the normal pressor potential contained in the NA stores.

It seems interesting that a similar process of NA depletion might occur in the brain since a replacement of NA by α-M-NA has been reported[2] – the amounts of which corresponded roughly to the missing NA – while there is no apparent decrease in the catecholamine content as determined by a biological estimation procedure (SMITH[1]). Our results, obtained from heart extracts, are in agreement with the observation made by SMITH and substantiate, thus, the suggestion that the presence of α-M-NA might interfere in the biological but not in the fluorimetric assay of NA[2].

The time course of the occurrence of α-M-NA in the heart (Figure 2) is correlated with a corresponding depletion of NA in such a manner that the total pressor activity remains unchanged throughout. This supports the view that the NA depletion is a direct consequence of the α-M-NA uptake and might therefore be in keeping with a displacement phenomenon, as observed first by CARLSSON[2] from studies of brain metabolism of α-M-DOPA as well as of α-MMT.

However, CARLSSON's displacement hypothesis is in conflict with the finding that the catecholamine loss produced in the brain and by the heart by α-MMT administration is not balanced stoichiometrically by the levels of α-methyl-*meta*-tyramine or metaraminol taken up and retained by these organs[6,7]. It seems at present not clear why there would be such a discrepancy between the mechanism of NA depletion in the guinea-pig heart produced by α-M-DOPA and α-MMT, since the latter was also found to exert its depleting effect by means of its decarboxylation products.

Résumé. L'α-méthyl-DOPA, même à doses élevées et/ou répétées, ne modifie pas significativement l'activité vasopressive totale – estimée sur la pression artérielle du rat décérébré et démédullé – des amines cathéchiques cardiaques. La quantité de noradrénaline libérée du coeur de cobaye 1 h à 6 jours après administration d'α-méthyl-DOPA y est remplacée par une quantité équipressive d'α-méthyl-noradrénaline.

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RNA and DNA Metabolism in Liver Cells of Normal and Cancer-Bearing Mice

Nucleic acid metabolism has been studied with labelled precursor adenine both *in vivo*[1] and *in vitro*[2] in the hepatic cells of mice and rat.

This paper presents studies *in vivo* on relative nucleic acid synthesis and replacement in the hepatic cells of nor-

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Table I. RNA incorporation in different organelles of cell

Interval	Normal mice			Cancer-bearing mice		
	Non-nucleolar region in nucleus	Nucleolus	Cytoplasm	Non-nucleolar region in nucleus	Nucleolus	Cytoplasm
15 min	7.7 ± 2.18	5.7 ± 2.24	6.8 ± 2.4	5.3 ± 2.00	3.0 ± 1.28	9.4 ± 2.1
1 h	21.5 ± 3.46	18.6 ± 3.74	19.5 ± 3.3	14.2 ± 3.34	12.7 ± 3.32	21.5 ± 4.7
3 h	12.3 ± 2.65	11.6 ± 3.00	29.9 ± 4.6	11.4 ± 2.99	12.9 ± 3.34	21.8 ± 4.4
8 h	8.1 ± 2.97	7.3 ± 3.07	39.6 ± 8.9	10.9 ± 2.95	9.3 ± 2.88	25.6 ± 5.7

mal and cancer-bearing mice on the basis of kinetic evidence with radioautographic technique.

Four normal C_3H mice and four mice of the same strain with mammary carcinoma were taken for this experiment; age and weight of all the animals were the same. They were injected intraperitoneally with 8-C14 adenine 25 μc/0.1 ml (sp. activity 5.06 mc/mM). The animals were sacrificed by cervical fracture at intervals of 15 min, 1 h, 3 h, and 8 h, respectively. A small bit of liver tissue was taken from each animal and fixed in Bouin's fluid overnight. After the usual histological procedure, 5 μ sections were cut and mounted on the gelatinized slides. After dewaxing, sections were treated with alcohol of different concentrations and finally with water. Then the slides were divided into three groups. The first group was treated with 5% cold TCA for 5 min at 2–4°C to remove any acid-soluble fraction containing adenine. The second group was treated for 7 min with NHCl at 60°C to remove RNA from the cells[3,4]; the resistant portion was assumed to be DNA. The third group was treated with 5% TCA at 90°C for 20 min to remove all nucleic acids.

The slides were then exposed with Kodak AR10 thin stripping plates for a period of 21 days using the technique of DONIAC and PELC[5]. All the photographic processes were done as described previously[6].

Nearly 300 liver cells from each treatment were studied and the distribution of the grains on different structures scored. Grain counts were done on those cells which had uniform nuclear diameter. Results are given in the Tables.

Discussion. Table I shows the incorporation of adenine into the hepatic cells of normal and cancer-bearing mice. It appears that, as early as 15 min, the nuclei and cytoplasm in both the series had simultaneous incorporation (Table I).

The nuclei of the normal series showed maximum uptake at 1 h which sharply declined with progress of time, while the nuclei of the cancer-bearing series also showed maximum uptake at 1 h with a very slow decrease as a function of time. With respect to cytoplasmic uptake, the normal series revealed a much higher and steady increase till 8 h than that of the cancer-bearing series. Sections extracted with TCA at 90°C showed no incorporation, proving the fact that adenine went into the nucleic acid. An attempt was also made to differentiate RNA and DNA by differential extraction. HCl extracted sections showed that all incorporation for the first 15 min went into RNA of nuclei (nucleolus + chromatin) and cytoplasm of both the series (Table I).

On comparing the nucleolar incorporation, it was observed (Table I) that the maximum uptake was noticed at 1 h both in the normal and cancer series, but the former had much higher uptake than the latter. As regards the movement of the labelled compound as a function of time in the two, it may be inferred that the nucleoli of the normal series showed high replacement synthesis while in the cancer-bearing series there was an equilibrium of retention mechanism. The non-nucleolar region in the nucleus of the normal series showed higher uptake and replacement than that of the cancer-bearing series. The RNA synthesis appeared to occur in all the components of cells of the two series (Table I) simultaneously.

The incorporation of adenine into both RNA and DNA in the same nuclei revealed that the incorporation of the precursor into RNA as compared to its incorporation in DNA for short intervals, up to 8 h, showed an inverse relationship (Table II). This relationship has also been observed by SISKEN[4] with pyrimidine precursor (orotic acid). However, nuclei of the cancer-bearing series showed slightly higher DNA synthesis than that of the normal series.

As regards RNA ratio of nucleus to cytoplasm (N/C) it has been observed that in the normal series the ratio decreased sharply from 1.9 to 0.4 within the first 8 h, showing thereby a steady diffusion from nucleus to cytoplasm.

In the cancer-bearing series, the ratio showed a stable value (nearly 1) indicating an equilibrium at least from 1–8 h (Table III)[7].

Table II. Percentage incorporation of RNA and DNA into the nuclear nucleic acid

Interval	Normal mice		Cancer-bearing mice	
	Percentage of RNA	Percentage of DNA	Percentage of RNA	Percentage of DNA
15 min	100	–	100	–
1 h	98.6	1.4	96.1	3.9
3 h	93.7	6.3	91.3	8.7
8 h	90.1	9.9	86.0	14.0

Table III. RNA ratio of nucleus to cytoplasm N/C as a function of time

Interval	Normal mice	Cancer-bearing mice
	RNA ratio of N/C	RNA ratio of N/C
15 min	1.9	0.8
1 h	2.0	1.2
3 h	0.8	1.1
8 h	0.4	0.8

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Résumé. Le métabolisme des acides nucléiques des cellules du foie de souris de la souche C_3H normales et atteintes d'une tumeur maligne, a été étudié à divers intervalles de temps avec de l'adénine marquée.

Dans le cas des souris normales, la proportion de RNA incorporée dans le noyau par rapport au cytoplasme se modifie fortement. Dans le cas des souris atteintes d'une tumeur maligne, ce rapport reste stable pendant la durée de l'expérience.

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Monocyte Participation in Connective Tissue Repair

A Quantitative Study in Diffusion Chambers Using Sex-Chromatin as a Marker

1. *Introduction.* Fate and function of leucocytes that have migrated into a field of inflammation induced by mechanical or chemical trauma present a large number of unsolved problems.

Leucocytes in tissue culture have been shown to proliferate and differentiate into macrophages on the one hand and into fibroblast-like cells on the other (ALLGÖWER[1], BLOOM[2], HULLIGER and ALLGÖWER[3]). The origin of these fibroblast-like cells developing in buffy coat cultures was investigated by comparing thoracic duct lymph containing 99% small and medium sized lymphocytes in the rabbit with blood leucocytes in tissue culture (HULLIGER[4]). In thoracic duct cultures, fibroblasts developed only very rarely. Large mononuclear cells (monocytes and large lymphocytes) were therefore suggested to be the stem-cells for the fibroblasts developing in blood cultures. Similar observations have been made by SHELTON[5] comparing rabbit lymph and blood cells in diffusion chambers.

Large mononuclear cells in human blood have also been shown to take up tritiated thymidine (BOND et al.[6]) thus demonstrating their reproductive capacity.

The fibroblastic nature of the spindle cells developing in tissue culture has been proved by their capacity to form collagen precursors as measured by hydroxyproline formation (ALLGÖWER and HULLIGER[7]). SHELTON[5] demonstrated hydroxyproline by leucocytes growing in diffusion chambers as well.

On the basis of experiments done *in vitro* and *in vivo*, ALLGÖWER[1] has suggested that the leucocytes migrating into a field of wound-healing participate in the fibrocytic population of repair tissue.

To obtain more information on the quantitative aspects of the leucocytic contribution to fibroblastic repair, experiments with combined fibrocytic and leucocytic cell populations in diffusion chambers were planned; the diffusion chambers were chosen, since the reactive inflammation around them would provide an inflammatory milieu. Leucocytes were combined with fibrocytes to include possible reciprocal influences between the different cell types. To follow the fate of both cell types quantitatively, a cellular label was needed.

The use of a metabolic label such as tritiated thymidine to follow the fate of leucocytes was not considered suitable, since reutilization of leucocytic DNA as shown by DUMONT[8], FICHTELIUS and DIDERHOLM[9], and BRYANT[10] might interfere with the interpretation of the data.

Sex chromatin was chosen as a suitable marker for our experiments; its advantages have been discussed (HULLIGER et al.[11]).

A model type of granulation tissue was prepared by enclosing known numbers of fibrocytes and of leucocytes of opposite sex in diffusion chambers. The diffusion chambers were implanted into the peritoneal cavity of rabbits, where an inflammatory exudate formed around the chambers and small blood vessels covered the outer membranes. After 2–3 weeks the percentage of fibrocytic nuclei deriving from leucocytes was counted, using sex chromatin as a marker.

2. *Material and Methods.* (a) *Tissue.* Subcutaneous connective tissue was excised from the abdomen of rabbits weighing 2–3 kg. The tissue was chosen from the thin, almost avascular connective tissue sheet between skin and muscle. The tissue sheets were spread and cut into square pieces of 0.5–1 mm side length. One piece was explanted in a diffusion chamber either alone or together with a piece of buffy coat. At the same time 4 to 6 randomly chosen pieces were explanted in a plasma clot on coverslides and immediately fixed in 95% alcohol to determine the cell types being present in the explant.

Buffy Coat: Blood was obtained by carotid artery cannulation from a rabbit of sex opposite to that of the donor of the connective tissue. Buffy coat was prepared by centrifugation at 2000 RPM for 8 min in a refrigerated centrifuge using siliconized tubes. The supernatant plasma was pipetted off without disturbing the buffy coat. The remaining blood with buffy coat was centrifuged a second time at 2000 RPM, and then incubated at 37° until clotting of the buffy coat was complete. The buffy coat was cut into small pieces whose sides measured 1–2 mm. 4 to 6 randomly chosen pieces were used for the determination of the initial cell number.

(b) *Diffusion chambers* were prepared according to the technique described by ALGIRE[12] using two lucite rings of 18 and of 13 mm diameter that would fit into one another. Millipore[13] filters (pore size 0.45 μ) of the same size were glued to each ring with a special glue[14]. The rings with the filters were dried at room temperature for 1–2 h. The tightness of the filters on the rings was examined by dipping the rings into 70% alcohol, where places that were not glued tightly rolled up. The rings were then sterilized under an ultraviolet lamp overnight.

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