Summary

A series of cyclohexylamine derivatives methylated both on nitrogen atom and in position 1, 2 and 6 of the cyclohexane ring have been prepared. Some of them showed considerable hypotensive and ganglioplegic activity. The most interesting compound is N, N, 1, 2, 2-pentamethylcyclohexylamine ('penhexamine') whose activity compares favourably with mecamylamine or dimecamine.

Albumin Synthesis in Regenerating Rat Liver Cells[1]

In a previous report dealing with the effects of the partial hepatectomy on the rat liver soluble proteins (GUIDOTTI and CLERICI[2]), evidence was given that these proteins from the regenerating liver present essentially the same electrophoretic pattern as those from the resting one. However, no reliable data on the albumin behaviour were obtained, because our technique failed to detect this protein.

Since the liver is the site of production of albumin in the rat (MILLER *et al.*[3,4], JENSEN and TARVER[5]), whose albumin concentration in serum is claimed to control liver regeneration (GLINOS and GEY[6], GLINOS[7]), an attempt has now been made to investigate the *in vitro* turnover of the above-mentioned protein in the resting and regenerating rat liver.

Male albino rats, Wistar strain, fed a standard diet, were hepatectomized according to HIGGINS and ANDERSON[8]. After 12 h fasting, normal and operated animals (72 h after hepatectomy) were killed and their livers perfused with saline solution in order to remove completely the blood proteins[2]. Livers were then taken out, sliced, and pooled to obtain 12 g of wet tissue slices. 6 g of tissue slices were added to a ml 100 flask containing 20 ml of a cold Ringer medium[9], and the whole content of the flask was quickly homogenized and lyophilized. The remaining 6 g of tissue slices were added to a second flask containing 20 ml of the above-mentioned medium supplemented with 20 μM of glycine-1-^{14}C (specific activity 1 $\mu c/\mu M$). After 2 h of incubation in air at 38°C, the flask was cooled in a ice bath; the content was then homogenized and lyophilized as indicated above.

Soluble proteins were extracted according to the technique of ADJUTANTIS[10] as modified by CIARANFI and DI SABATO[11]. 0·02 and 0·04 ml of a 7% water solution of proteins from each sample, and 0·02 ml of normal rat serum, were utilized for a paper electrophoresis carried out as described by the above mentioned authors[10,11]. All the steps of this procedure were performed in a cold room (+ 2°C).

Amidoschwarz-stained strips were photometrically scanned by means of an Elphor photometer, and the percentage of the protein content was evaluated by planimetry. The albumin-carrying section of each strip was separated from the other soluble proteins and hydrolized with HCl 5 *N* in sealed ampulles kept at 116°C for 16 h. The cloudy suspension obtained thereafter was centrifuged; the supernatant fluid, poured into suitable polythene planchets, was dried under a current of warm air. Radioactivity measurements were performed by means of a windowless flow counter; counts were corrected for self absorption.

After incubation, a protein peak, whose migration rate was similar to that of the serum albumin, was always detected in both the electrophoretic patterns from resting and regenerating rat liver slices. Therefore, this peak, constantly lacking in the electrophoretic patterns from non-incubated liver slices[2], was tentatively identified with albumin, newly released.

As for the evaluation of the amount of albumin released during incubation, some errors may arise from the following causes: (1) protein loss during the extraction procedure; (2) inaccuracy of photometric readings due to different affinity of the several proteins towards Amidoschwarz stain; (3) uncertain separation of the overlapping planimetric peaks, and (4) contamination of the albumin peak from α_1-globulin. It has been estimated, however by experience, that with reasonable care these errors do not exceed $\pm$ 10–15% of the found values. Radioactivity measurements, which were referred to a mg of albumin, are more reliable because not biased by the main error source, i.e. by number (1).

The results concerning the amount of albumin released during incubation and the incorporation rate of the labelled glycine into the same protein are presented in the Table.

It appears that, during incubation, less albumin is released from the regenerating rat liver, as compared to the resting one. However, the difference is not statistically significant ($P > 0{\cdot}05$).

Furthermore, the glycine incorporation rate into the albumin released from the regenerating liver significantly increases over the resting one ($P < 0{\cdot}01$).

[1] This investigation was aided by a grant from C.N.R., Roma, and was made during a tenure by one of us (G.G.) of a Scholarship from C.N.R., Roma.

[2] G. GUIDOTTI and E. CLERICI, Exper. *14*, 341 (1958).

[3] L. L. MILLER, C. G. BLY, M. L. WATSON, and W. F. BALE, J. exp. Med. *94*, 431 (1951).

[4] L. L. MILLER, C. G. BLY, and W. F. BALE, J. exp. Med. *99*, 133 (1954).

[5] D. JENSEN and H. TARVER, J. gen. Physiol. *39*, 567 (1956).

[6] A. D. GLINOS and G. O. GEY, Proc. Soc. exp. Biol. Med., N.Y. *80*, 421 (1952).

[7] A. D. GLINOS, Fed. Proc. *15*, 76 (1956).

[8] G. M. HIGGINS and R. M. ANDERSON, Arch. Path. *12*, 186 (1931).

[9] G. GUIDOTTI, E. CLERICI, and E. BAZZANO, Min. nucl. *2*, 14 (1958).

[10] G. ADJUTANTIS, Nature *173*, 539 (1954); *174*, 1054 (1954).

[11] E. CIARANFI and G. DI SABATO, Pubbl. Staz. zool. Napoli *28*, 296 (1956).

Table

Type of tissue used	Number of experiments	Albumin release (mg/g of wet tissue/h)	Glycine-1-^{14}C incorporation into albumin (counts/min/mg)
Resting liver	7	0·23 ± 0·04*	23·8 ± 3·0
Regenerating liver	6	0·18 ± 0·01	55·3 ± 4·6

* Mean ± S.E.

This behaviour may suggest that the albumin turnover in the regenerating liver is faster than in the resting one and that the partial hepatectomy, as far as the albumin synthesis is concerned, does not impair the liver function.

The above results rule out the assumptions[12] which attribute the lowering of the serum albumin levels in the hepatectomized animals to a decreased production rate of albumin from the liver cells. On the contrary, they may suggest that the serum albumin concentration decreases after operation because of an increased rate of serum albumin utilization by the liver itself, in order to rebuild its surgically removed parenchima.

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Institute of General Pathology, University of Milan (Italy), September 29, 1958.

Riassunto

Gli autori hanno studiato *in vitro* il problema della sintesi dell'albumina in sezioni di fegato normale e rigenerante di ratto. La quantità di albumina prodotta durante l'incubazione non presenta notevoli variazioni nelle due situazioni studiate. Al contrario l'incorporazione di glicina marcata nella albumina del fegato rigenerante risulta nettamente aumentata rispetto a quella del fegato normale. Tale comportamento viene interpretato come espressione di un ricambio albuminico più elevato nel fegato rigenerante che nel normale.

[12] N. H. MARTIN and A. NEUBERGER, Brit. med. Bull. *13*, 113 (1957).

The Local Oedema-Producing Effect of Disodium Ethylendiamine Tetraacetate (EDTA-Na_2)

In a series of experiments CHAMBERS *et al.*[1], and ZWEIFACH[2] found that one of the substances essential to the functional and structural stability of the capillary wall was calcium present in the interendothelial cement substance. Since, at the pH of the organism, calcium is bound by EDTA-Na_2 more effectively than by sodium citrate, it seemed worth while to study the effect of EDTA-Na_2 on capillaries.

Albino rats were injected subcutaneously with a single dose of EDTA-Na_2 *suspension* (60 mg) in the interscapular region. After 7 to 12 h a local, tumor-like oedema developed at the site of injection (Fig. 1). Histologically it showed oedematous structure, poor in cells. Suspensions of sodium citrate (200 mg), sodium acetate (200–400 mg), and pulverised gypsum (200 mg) failed to produce oedema.

The oedema-producing effect of EDTA-Na_2 was also examined with the more sensitive method of ROWLEY and BENDITT[3], by which the severity of the oedema could be measured quantitatively. The oedema produced by a substance injected subcutaneously into the dorsa of the hind paws of rats was demonstrated by intravenously injected Evans blue, which stained the oedematous tissues blue. For details see Ref. [3].

[1] R. CHAMBERS *et al.*, Acta Unio intern. contra Cancrum *6*, 696 (1949).

[2] B. W. ZWEIFACH, Connective Tiss. Conf. Fifth Trans. (J. Macy Jr. Found., New York 1954), p. 42.

[3] K. ROWLEY and E. BENDITT, J. exp. Med. *103*, 399 (1956).

Figure 2 shows that a *solution* containing 370 γ of EDTA-Na_2 or 200 γ of histamine induces practically the same degree of blue colouring, i.e., the two substances are similarly effective in producing oedema. Ca-EDTA-Na_2, on the other hand, not being able to bind calcium, proved ineffective. Figure 3 shows the quantitative data obtained in the course of the experiment. Histamine and EDTA-Na_2 are nearly similar in their effects, whereas Ca-EDTA-Na_2 (410 γ), or sodium citrate (200 γ) do not produce oedema. The antihistaminic agents Antistin and Synopen, or Dibenamine, which is a 5-hydroxytryptamine antagonist[4], do not inhibit oedema produced by EDTA-Na_2.

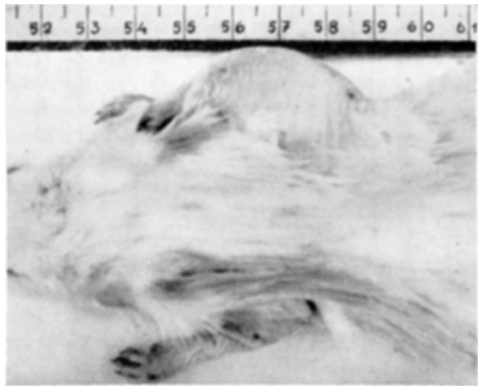

Fig. 1.—Local oedema developed 1 h after the subcutaneous injection of 60 mg of an EDTA-Na_2 *suspension* (in 1 ml of saline)

Discussion. According to our observations subcutaneously injected EDTA-Na_2 produces oedema at the site of injection. The high potency of *suspensions* of EDTA-Na_2 is due to its poor solubility at the pH of the organism resulting in a prolongation of the effect. Since the removal of calcium from the capillary wall increases permeability[2], the oedema-producing property of EDTA-Na_2, which is essentially greater than that of sodium citrate, might be

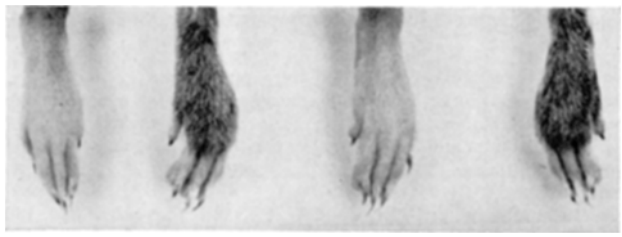

Fig. 2.—Blueing of rat paw skin 2 h after the injection of EDTA-Na_2 (370 γ), histamine (200 γ), and Ca-EDTA-Na_2 (410 γ), in 0·1 ml saline, respectively. Control: 0·1 ml saline

due to a more stable binding of calcium. The ineffectiveness of Antistin, Synopen, and Dibenamine to inhibit EDTA-Na_2-produced oedema shows the direct effect of EDTA-Na_2. The fact that Ca-EDTA-Na_2 is not an oedema-producing substance may be interpreted to mean that not the EDTA-Na_2 molecule *per se*, but its calcium-binding capacity is responsible for the oedema. According to CHAMBERS and ZWEIFACH[5], magnesium, which is also

[4] J. H. GADDUM and K. A. HAMEED, J. Pharmacol. *9*, 24 (1954).

[5] R. CHAMBERS and B. W. ZWEIFACH, J. cell. comp. Physiol. *15*, 255 (1940).