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.

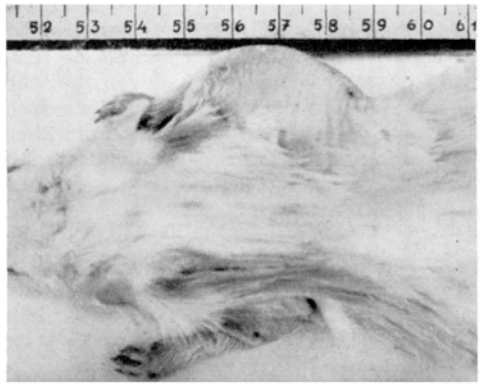

Fig. 1.—Local oedema developed 1 h after the subcutaneous injection of 60 mg of an EDTA-Na_2 *suspension* (in 1 ml of saline)

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

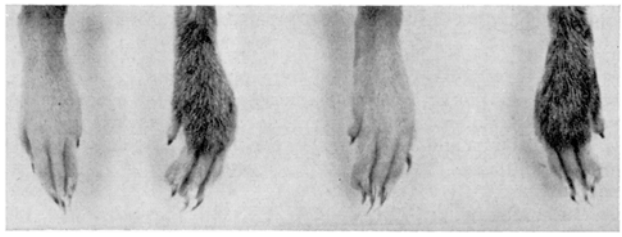

Fig. 2.—Blueing of rat paw skin 2 h after the injection of EDTA-Na_2 (370 γ), histamine (200 γ), and Ca-EDTA-Na_2 (410 γ), in 0·1 ml saline, respectively. Control: 0·1 ml saline

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).

bound by EDTA-Na_2, does not play a role in the processes taking place in the capillary wall.

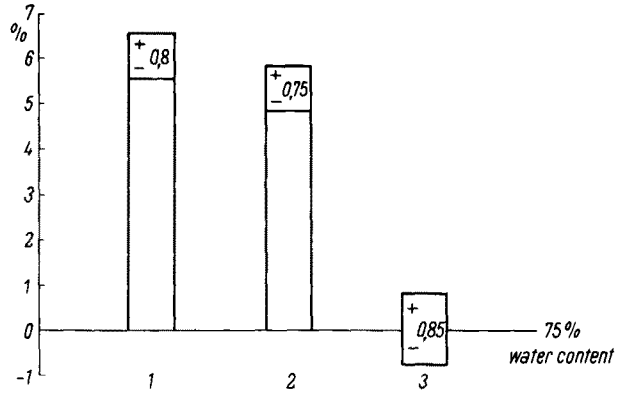

Fig. 3.—The changes in water content of the paw skin of rats. 0% in the diagram indicates the normal water content of skin (75%). Column 1: Histamine (200 γ, subcut.) (6). Column 2: EDTA-Na_2 (370 γ, subcut.) (12), and EDTA-Na_2+Antistin (400 γ, subcut.), or Synopen or Dibenamine (800 γ, intraven.) (18). Column 3: Ca-EDTA-Na_2 (410 γ, subcut.) (6), sodium citrate (200 γ, subcut.) (6) or saline (0·1 ml, subcut.). All substances were dissolved in 0·1 ml saline. The number of animals used in each group is given in brackets.

In 1954 MEYER[6] assumed that EDTA-Na_2 might, in some way, influence capillary function. Our observations would seem to corroborate this concept and suggest that the local oedema-producing effect of EDTA-Na_2 is due to a binding of the calcium in the interendothelial cement substance of the capillaries.

Thanks are due to Dr. A. KÓRÖSSI and to Dr. S. SAJÓ, Budapest, for their valuable help and suggestions during the course of this study.

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Zusammenfassung

Die Lösungen oder Suspensionen von Äthylendiamintetraacetatdinatrium (EDTA-Na_2) erzeugen an der Applikationsstelle Ödeme, welche wahrscheinlich der Bindung von Kalzium in der interendothelialen Zementsubstanz der Kapillaren zuzuschreiben sind.

[6] K. MEYER, Connective Tiss. Conf. Fifth Trans. (J. Macy Jr. Found., New York 1954), p. 73.

The Citric Acid Content of Embryos of *Rana esculenta*

It was previously shown that the mode of determination of the developing crystalline lens in *Rana esculenta* varies with temperature, and that the lactic acid content of gastrulae and neurulae is lower at 12°C than at 25°C[1,2]. Although the effect of temperature on the citric acid content was found to be small, the experimental results may have some value since the literature contains but few data on this subject.

Citric acid content of *Rana esculenta* embryos (µg/100 embryos)

Stage	25°C (*n*)		12°C (*n*)		Average (*n*)	
10	—	—	44	(1)	44	(1)
10½	42	(1)	—	—	42	(1)
11	—	—	46	(1)	46	(1)
11½	—	—	—	—	—	—
12	—	—	—	—	—	—
12½	67	(1)	—	—	67	(1)
13	—	—	—	—	—	—
13½	—	—	—	—	—	—
14	44	(2)	58	(1)	49	(3)
14½	—	—	—	—	—	—
15	—	—	—	—	—	—
15½	—	—	—	—	—	—
16	38	(2)	69	(2)	54	(4)
16½	50	(1)	—	—	50	(1)
17	—	—	50	(1)	50	(1)
17½	56	(2)	68	(1)	60	(3)
18	46	(2)	96	(1)	63	(3)
18½	—	—	—	—	—	—
19	71	(2)	59	(1)	67	(3)
19½	71	(1)	—	—	71	(1)
20	63	(2)	83	(1)	70	(3)
20½	78	(6)	81	(5)	79	(11)
21	75	(4)	73	(3)	74	(7)
21½	—	—	—	—	—	—
22	91	(4)	123	(2)	100	(6)
22½	107	(2)	86	(1)	100	(3)
23	94	(4)	81	(3)	88	(7)
23½	168	(2)	—	—	168	(2)
24	155	(2)	92	(3)	123	(5)
24½	125	(1)	68	(1)	97	(2)
25	128	(7)	67	(3)	111	(10)

Stages according to SHUMWAY.
(*n*) number of duplicate experiments.

Eggs were reared in tap water either at 12°C or at 25°C. For citric acid determinations, 40 to 200 embryos were ground in 10% trichloroacetic acid and after centrifugation the supernatant was assayed by the method of NATELSON *et al.*[3]. One extraction was sufficient. Within the range from 10 to 40 µg, the standard deviation was 3%. Mostly the amount of citric acid was calculated per embryo in order to compare identical stages from the same batch, reared at different temperatures. In some cases, fresh or dry weight was determined. Stages were indicated according to SHUMWAY[4].

The results are presented in the Table and Figure 1. The quantity of citric acid per embryo varied largely between different batches, probably mainly because of size differences. However, the general trend is clear: after a slow increase between stages 10 and 17, the content rises more rapidly until stage 22, whereafter it increases still more at 25°C, but declines at 12°C. Finally it decreases at both temperatures. Only in one period, a difference between the two temperature groups could thus be found. In some experiments on larvae, reared at room temperature until some days after stage 25 and fed with vegetal material, dry weight was determined. First, 400 larvae were used for a (duplicate) assay: each larva weighed 0·53 mg and contained 0·71 µg (134 mg%) citric acid. The next day another 400 larvae of the same group, which had been starved for one day, had an average weight of 0·46 mg and contained 0·62 µg (111 mg%). Four experiments were made on ovaries of *Rana temporaria*, shortly before

[1] G. TEN CATE, Nederl. Tijdschr. Geneesk. *90*, 1695 (1946).
[2] G. TEN CATE, Verh. Kon. Nederl. Akad. Wetensch. Amsterdam *51*, Nr. 2 (1956).
[3] S. NATELSON, J. B. PINCUS, and J. K. LUGOVOY, J. biol. Chem. *175*, 745 (1948).
[4] W. SHUMWAY, Anat. Rec. *78*, 139 (1940).