Studies of metabolism of parenterally administered EDTA in animals and humans(10, 11) have demonstrated a biological half-life of approximately 30 minutes. When coupled with the present work this would suggest that the synthetic chelates would be limited in iron combination *in vivo* to those areas of the body where they would be in direct competition with the iron-binding protein for "free" ferric or ferrous ions. The tissue iron storage areas are considered to be in this category(12). Here the synthetic chelate could bind iron in competition with the unsaturated iron-binding protein to an extent governed by competitive iron-binding ability, effective compound concentration, size of iron storage, and metabolic fate of the iron chelate.

Summary and conclusions. 1. The iron-binding ability of a series of synthetic chelating agents has been compared to that of siderophilin in rabbit serum. 2. The chelate compounds are unable to remove iron from siderophilin in rabbit serum. 3. Under the same conditions siderophilin is unable to remove iron from the more tightly bound iron chelates. 4. EDTA and HOEDTA, at iron-binding ratios of 20-25 1 and 3-5 1 respectively to that of the protein, compete on equal basis for iron added to rabbit serum.

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Connective Tissue II. Effect of Age and Sex upon Lipid Composition of Tissues of Rat.* (25628)

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Further to correlate the behavior of lipids and protein in connective tissue, the present study was undertaken to determine the effect of age and sex upon: (1) Free cholesterol, (2) Total cholesterol, (3) Phospholipids, and (4) Triglycerides in the connective tissue of various structures of the rat.

Procedures. Male and female rats of the Wistar strain, from 4 weeks to 2½ years old were used in this study. One-tenth to 0.5 g of tail tendon, aorta, skin and uterus, respectively, were cut into small pieces and heated to boiling with 20 ml portions of 1:1 acetone-alcohol, 3 times, and with 3:1 ether-alcohol, twice. After each extraction, the extracting tubes were centrifuged at 2500 rpm for 5 minutes. In each instance, the supernatant was filtered. The combined extracts were evaporated on a steam bath to dryness and made up to a known volume with a 2:1 methyl alcohol-chloroform mixture. Aliquots were taken for determination of free and total cholesterol, phospholipids and total lipids. Free cholesterol was precipitated as digitonide and determined colorimetrically by the method of

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TABLE I. Effect of Age and Sex upon Lipid Composition of Skin of Rat (mg/g of Tissue).

	Age (wk)		Cholesterol Free	Cholesterol Total	Phospholipids	Triglycerides
♂	4-5 (30) A		1.8 ± .5 BCDE <.001	3.3 ± .6	7.6 ± 1.1 BCDE <.001	45.2 ± 17.8 BC <.001
	7-11 (24) B		1.3 ± .2 CDE <.001	3.1 ± .6	5.7 ± 1.4 CDE <.001	98.0 ± 43.3 D <.05 E <.01
	17-26 (12) C		.9 ± .1 DE <.05	2.8 ± .7	*3.3 ± 1.6*	111.3 ± 51.0 E <.01
	30-34 (12) D		*.8 ± .1*	2.3 ± .5	3.5 ± 1.3	*69.0 ± 30.5*
	130 (10) E		.8 ± .1	*3.1 ± 1.0*	3.0 ± .6	*52.5 ± 16.1*
♀	4-5 (30) A		2.0 ± .2 BCDE <.001	3.2 ± .4 C <.05 DE <.001	8.0 ± 1.6 DE <.001	55.0 ± 23.0 BCDE <.001
	7-11 (20) B		1.4 ± .3 CDE <.001	2.7 ± .5 C <.01 DE <.001	6.0 ± 1.1 DE <.001	127.0 ± 64.0
	17-26 (15) C		1.0 ± .1	2.2 ± .6	*6.2 ± 1.4* D <.001 E <.01	175.0 ± 42.0
	30-34 (16) D		*1.0 ± .1*	2.0 ± .5	3.4 ± .9	*179.0 ± 54.0*
	130 (9) E		.9 ± .1	*1.7 ± .1*	3.4 ± 1.4	*138.0 ± 41.0*

Figures in parentheses represent No. of animals and corresponding No. of determinations unless otherwise specified.

± indicates stand. dev. of mean.

P value relating to age differences, when within 5% level of confidence, is listed beneath results of actual determination and represents a comparison between that figure and that of age groups designated by letter or letters preceding the value.

Italicizing designates significant P values within 5% level of confidence relating to sex differences.

Sperry and Webb(1). The ester cholesterol was hydrolyzed and determined in the free state by the method just mentioned. Phospholipids were determined by the method of Fisk and Subbarow(2); total lipids by the Bragdon dichromate oxidation procedure(3); and triglycerides calculated by difference as described by Bragdon(3).

Results. The effects of age and sex upon composition of lipids in the skin, tendon and uterus of rats are recorded in Tables I to III, inclusive. In tendon no significant changes were observed beyond the 17 - 26 week period; therefore, data beyond this have been omitted from the tables. For the same reason uterine data beyond the 8th month have been omitted from Table III. The only significant changes observed in aortic lipids are concerned with esterified cholesterol. Therefore, no table has been constructed on the data in this structure. In the male, esterified cholesterol ranged from .11 ± .05 mg/g at 4 - 5 weeks to .22 ± .03 at 2½ years. For the female, corresponding figures were .01 ± .02 to .22 ± .09. In the male, most of this change appeared between 7 and 11 weeks, whereas in the female it was not observed until the 17th - 26th week. Initial figures for the other lipids in the aorta of the male rat were in mg/g of tissue: Free cholesterol 1.5 ± .5, total cholesterol 1.6 ± .5, phospholipids 9.5 ± 5.8 and triglycerides 34.8 ± 22.0. The female figures did not differ significantly from those quoted for the male. In making these determinations from the aorta, all specimens were pooled from 2 - 3 rats and from 2 - 9 determinations were made at each of the 5 age levels mentioned in Table I.

Variations in tissue lipids with age included:

a. In skin, a decrease in free cholesterol in both sexes, and also of total cholesterol in the

TABLE II. Effect of Age and Sex upon Lipid Composition of Tendon of Rat (mg/g of Tissue).

	Age (wk)		Cholesterol Free	Cholesterol Total	Phospholipids	Triglycerides
♂	4-5 A	(15)*	2.5 ± 1.1 B <.02 CDE<.001	2.5 ± 1.1 B <.01 CD<.001	8.9 ± 3.3 B <.01 CD<.001	20.6 ± 13.7
	7-11 B	(6)*	1.2 ± .5 D <.05	1.2 ± .4 D <.05	4.2 ± 2.8	13.2 ± 4.7
	17-26 C	(6)	.7 ± .4	.8 ± .1	2.3 ± .7	8.6 ± 1.5
♀	4-5 A	(12)*	1.9 ± .2 BCD <.001	2.1 ± .5 CD <.001	7.1 ± 1.6 BCD <.001	36.0 ± 25.2
	7-11 B	(7)*	1.3 ± .4 D <.01	1.4 ± .4 D <.01	4.3 ± 1.1 CD <.001	21.3 ± 11.4
	17-26 C	(5)	.7 ± .5	.8 ± .5	2.3 ± .7	11.2 ± 2.1

See legend, Table I.

* Each determination consists of a pooled sample from 2-3 rats.

TABLE III. Effect of Age upon Lipid Composition of Rat Uterus (mg/g of Tissue).

		Female			
Age (wk)		Cholesterol Free	Cholesterol Total	Phospholipids	Triglycerides
4-5 A	(8)*	2.4 ± .3	2.7 ± .4	12.5 ± 2.9 D <.001	27.2 ±15.5
7-11 B	(7)*	2.4 ± .8	2.8 ±1.0	12.0 ± 3.2 D <.001	15.1 ± 8.5
17-26 C	(5)	2.2 ± .0	2.5 ± .2	9.9 ± 2.7	9.9 ± 2.6
30-34 D	(8)	2.2 ± .4	2.4 ± .5	6.3 ± 1.4	15.3 ± 5.6

See legend, Tables I and II.

female; a decrease in phospholipids; and an increase in triglycerides in both sexes;

b. in tendon, an increase in ester cholesterol and a decrease in free and total cholesterol and in phospholipids in both sexes; and a decrease in triglycerides in the female;

c. in aorta, an increase in cholesterol esters; and

d. in uterus, a decrease in phospholipids. Sex differences were observed only in skin. Values for free cholesterol, phospholipids and triglycerides were higher in female than in male skin; the reverse was true for the value of cholesterol esters.

Comments. On analysis of whole rat carcass, Williams and his associates found changes between birth and 70 days of age similar to those recorded here(4). However, if determinations were limited to parenchymatous structures, they noted that cholesterol and phospholipids were increased(5). In the muscle of several species of animals, cholesterol has been observed to decrease with age (5,6). In bone marrow, several workers have found a decrease in lecithin with age(7,8,9). An increase with age in the lipids of renal epithelium has been reported(10).

Our findings of higher values for free cholesterol, phospholipids and triglycerides in the skin of female than in that of male rats agree well with those of Taylor and his associates(11). In the aortic tissues of the rat, the lack of a significant increase of cholesterol or neutral fat observed in these studies is in sharp contrast to corresponding data for chicken(12), horse(13) and man(14). Unlike skin and tendon, there is no decrease as a function of age in the cholesterol of the aorta.

Summary. 1. Changes as a function of age in values for free cholesterol, total choles-

terol, phospholipids and triglycerides in lipid extracts of rat tissues occurred between 4 weeks and 8 months of age, with no further changes, except in skin, at 2 years of age. 2. Sex differences in lipid composition occurred only in skin.

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Inhibition of Release of Intracellular Potassium by Non-Narcotic Analgesic Agents. (25629)

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It has been shown that all types of pain-producing stimuli also release intracellular potassium (K) and that inhibition or reversal of K release is associated with decreased pain sensation(1). This new approach to pain might have some bearing on the action of analgesic agents. Therefore the present investigation is an attempt to determine whether non-narcotic analgesic agents inhibit release of intracellular K.

Two *methods* were used to produce a controlled release of intracellular K. The first one is distention of the intestine of guinea pigs. This procedure is well established as a painful stimulus(2,3). However K release under these conditions has not been shown previously. The other procedure used was exposure of washed erythrocytes to cold. In this case K release is a well established fact (4,5), but the relation to pain is purely by analogy. In the first procedure a guinea pig is killed by a knock on the head. The abdominal cavity is opened and 10 pieces of intestine, 2½ inches long, are cut. Intestinal chyme is gently pressed out. Each piece is washed in physiological saline and weighed (wet weight). One end of the strip is tied off with heavy nylon thread. The other end is tied to a glass rod. These rods have been prepared previously from glass tubing, external diameter 6.2 mm and internal diameter 5 mm. The rods are tapered and a small bulb is blown at the end to provide firm attachment for the strip (Fig. 1). The preparations are then transferred into 50 ml test tubes filled with 20 ml of K free Tyrode solution. Control experiments showed that there is a gradient of increasing K release down the intestinal tract (Table I). Therefore experimental preparations containing the analgesic agent are alternated with control tubes. The whole set of tubes is then transferred to an automatically stirred water bath kept at 37°C. After a control period of 30 minutes the intestine is distended with Tyrode solution (Fig. 1) until a steady fluid pressure of 2.5 cm is obtained. Samples are taken immediately after distention, one hour later and 2 hours

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