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DISPOSITION OF [^{35}S]-HEPARIN IN THE RAT

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[^{35}S]-Heparin is rapidly taken up by tissues after intraperitoneal injection and rapidly degraded. Skin, aorta and lung showed an especially high affinity for [^{35}S]-heparin. In these organs, as well as in adrenal gland, stomach, heart and brain, the isotope was found preponderantly as dialysable metabolite(s) of heparin. Another group of tissues (large intestine, fat, kidney and liver) was characterized by a smaller proportion of dialysable material. Tissues that showed a high affinity for exogenous heparin appeared to catabolize it rapidly. It is also noted that some of the radioactive material present in tissues after the injection of [^{35}S]-heparin could come from the uptake of [^{35}S]-sulphate resulting from the degradation of [^{35}S]-heparin. The incorporation of [^{35}S]-sulphate into the sulphomucopolysaccharides of some tissues is recorded.

Both in its anticoagulant action and its effect on fat metabolism, heparin has a quick onset and a brief duration of action (Robinson & French, 1960), characteristics which could be explained by rapid fixation and catabolism of the drug by tissues. It was thought that observations on the distribution of exogenous [^{35}S]-heparin in tissues might help to explain the short-lived effects of the drug, and these findings are presented here.

Studies on the distribution of [^{35}S]-heparin are complicated by the fact that it is rapidly degraded to [^{35}S]-sulphate (Danishefsky & Eiber, 1959), which is reabsorbed by the kidney (Berglund, 1960), and this [^{35}S]-sulphate could then be taken up by tissues and incorporated into their sulphomucopolysaccharides, which cannot easily be distinguished from the original [^{35}S]-heparin. Therefore, to facilitate the interpretation of observations in the distribution of [^{35}S]-heparin, concomitant studies on the incorporation of [^{35}S]-sulphate into the sulphomucopolysaccharides of tissues were also carried out, and these are included in this paper.

METHODS

Preparation of [^{35}S]*-heparin.* This was prepared from a murine mast cell tumour, P-815-X-1, which was grown in the ascitic form for seven days in mice (Green & Day, 1960). Sixteen to 24 hr before killing each mouse, 1 mc of [^{35}S]-sulphate (obtained from the Oak Ridge National Laboratory) was injected intraperitoneally, and the [^{35}S]-heparin was isolated, as described by Green & Day (1960), by proteolysis, dialysis, precipitation with acetone, and removal of residual protein by extraction with trichlorotrifluoroethane, which also removes the cerebroside sulphate found in mast cells and other tissues (Green & Robinson, 1960). The crude heparin was purified by chromatography on a cellulose ion-exchange column (Green, 1960). Biological activity was equivalent to 120 u./mg ; its specific activity was 15,000 cpm/mg.

Distribution of [^{35}S]-heparin. One mg of [^{35}S]-heparin was injected intraperitoneally into rats. At stated intervals the animals were decapitated. Blood was collected in 8% sodium citrate and centrifuged. The lumina of heart, aorta, stomach and intestine were gently washed clean with a jet of water. Tissues and organs were blotted and weighed. They were homogenized in cold water, and an aliquot of the suspended tissue was placed in the scintillation counter for the determination of total radioactivity. Sulphomucopolysaccharide, calculated as heparin, was extracted from the rest of the tissue as described above and its radioactivity measured in a liquid scintillation counter. The difference between total radioactivity in the tissues and the radioactivity in heparin was attributed to non-sulphomucopolysaccharide. Experiments on lung, stomach, large intestine and liver showed that 97 to 100% of the radioactivity in the non-sulphomucopolysaccharide fraction was dialysable.

Incorporation of [^{35}S]-sulphate into sulphomucopolysaccharides. To study the incorporation of [^{35}S]-sulphate into the sulphomucopolysaccharide fraction in tissues of the rat, 1.0 mc of [^{35}S]-sulphate was injected intraperitoneally. Six hours later the tissues were removed, weighed and the sulphomucopolysaccharides extracted by the same procedure used to isolate heparin.

RESULTS

After the injection of [^{35}S]-heparin, skin, aorta and lung contained more radioactivity, per gram of wet tissue, than any other organ examined. From the results presented in Table 1 the tissues may be placed into two groups.

TABLE 1

DISTRIBUTION IN RAT OF RADIOACTIVITY AS HEPARIN (OR SULPHOMUCOPOLYSACCHARIDE, SMPS) AND AS NON-SULPHOMUCOPOLYSACCHARIDE (NON-SMPS) AT INTERVALS AFTER THE INTRAPERITONEAL INJECTION OF [^{35}S]-HEPARIN

	% of injected radioactivity/g or ml.					
	0·5 hr		2 hr		6 hr	
Tissue	SMPS	Non-SMPS	SMPS	Non-SMPS	SMPS	Non-SMPS
Plasma	0·16	0·10	0·06	0·40	0·00	0·33
Skin (foot)	0·00	36·2	0·00	26·4	0·00	8·4
Aorta	0·00	28·0	0·00	6·3	0·00	0·0
Lung	0·50	16·8	0·00	0·0	0·00	0·0
Adrenal gland	0·00	5·3	0·00	4·4	0·00	2·5
Stomach	0·96	1·4	0·80	1·2	0·57	0·81
Heart	0·26	0·47	0·05	0·38	0·00	0·0
Brain	0·00	0·30	0·00	0·10	0·00	0·0
Large intestine	1·4	0·43	0·33	1·2	0·23	0·07
Fat (perirenal)	0·83	0·03	0·53	1·1	0·17	0·14
Kidney	0·47	0·30	0·43	0·67	0·30	0·23
Liver	0·43	0·33	0·24	0·72	0·17	0·03

One group of tissues (skin, aorta, lung, adrenal gland, stomach, heart and brain) was marked by the accumulation, even at the earliest time interval, of more non-sulphomucopolysaccharide than sulphomucopolysaccharide. In fact, sulphomucopolysaccharide was not found in skin, aorta, adrenal gland or brain. In all tissues within this group the level of radioactivity in both fractions diminished with time. All the non-sulphomucopolysaccharide was dialysable material, including inorganic sulphate. The relatively high level of non-sulphomucopolysaccharide material in these tissues could arise either from a high uptake of inorganic [^{35}S]-sulphate from plasma or from the destruction *in situ* of [^{35}S]-heparin. The former explanation cannot account for these findings, for at 2 and 6 hr plasma levels of non-sulpho-

mucopolysaccharide were higher than at 0.5 hr, yet the amount of dialysable radioactive material in these organs had fallen. It is more likely that these organs are able to catabolize [^{35}S]-heparin at a rapid rate.

In another group of tissues (large intestine, perirenal fat, kidney and liver) more isotope was initially found in the sulphomucopolysaccharide fraction than as non-sulphomucopolysaccharide ; however, within 2 hr after the injection of [^{35}S]-heparin, the radioactivity in the sulphomucopolysaccharide fraction had fallen and that in the non-sulphomucopolysaccharide had concomitantly increased. This change at 2 hr in the relative levels of the two fractions could be attributed either to the uptake of [^{35}S]-sulphate from plasma or to breakdown of [^{35}S]-heparin *in situ*. By 6 hr, both the sulphomucopolysaccharide and the non-sulphomucopolysaccharide in this group of tissues had decreased, but there was a proportionally greater fall in the non-sulphomucopolysaccharide. The disproportionate decrease in the non-sulphomucopolysaccharide raises a question as to whether some of the non-sulphomucopolysaccharide (primarily inorganic sulphate) is being reincorporated into sulphomucopolysaccharide. Such a hypothesis implies that this group of tissues has a greater capacity to incorporate precursors of sulphomucopolysaccharide into sulphomucopolysaccharide than the previous group of tissues. Table 2 shows that

TABLE 2

INCORPORATION OF [^{35}S]-SULPHATE INTO THE SULPHOMUCOPOLYSACCHARIDE FRACTION OF TISSUES OF RAT 6 HR AFTER THE INJECTION OF [^{35}S]-SULPHATE (1 mc)

Tissue	cpm/g fresh tissue
Large intestine	960,000
Stomach	670,000
Kidney	370,000
Lung	230,000
Liver	230,000
Adrenal gland	210,000
Aorta	120,000
Brain	81,000
Heart	66,000
Fat (perirenal)	26,000

although the capacity of tissues to incorporate [^{35}S]-sulphate into the sulphomucopolysaccharide fraction is not precisely related to the disposition of sulphomucopolysaccharide in these tissues at 6 hr (Table 1) in three organs (large intestine, stomach and kidney) with a high capacity for synthesis of sulphomucopolysaccharide, exogenous sulphomucopolysaccharide persisted.

DISCUSSION

As early as 15 min after the administration of [^{35}S]-heparin, radioactivity is detectable in some organs (Eiber, Danishefsky & Borelli, 1958). Table 1 shows that by 30 min some of the [^{35}S]-heparin has been degraded. In fact, in one group of tissues (skin, aorta, lung, adrenal gland, stomach, heart and brain) most of the isotopic material was found to be dialysable 30 min after the injection of [^{35}S]-heparin ; within this group, skin, aorta, adrenal gland and brain showed only degraded material. Other tissues (large intestine, fat, kidney and liver) showed a preponderance of isotope in the fraction containing sulphomucopolysaccharide, almost

certainly as [^{35}S]-heparin, but even in these tissues dialysable material was present. The most reasonable interpretation of the findings would be that tissues vary in their capacity to take up and degrade heparin; and, from the relative levels of the two fractions, it appears that those tissues showing an affinity for heparin also catabolize it rapidly. Skin, which has recently been shown to degrade chondroitin sulphate-B (Davidson & Riley, 1960), and aorta were notable in this respect. The high affinity of aorta for heparin is interesting in view of the suggestion that heparin may release clearing factor lipase from the vascular wall (Robinson & French, 1960; Gore & Larkey, 1960).

Besides the affinity of tissues for heparin and their rates of degrading it, another factor that can influence the final concentration (and nature) of the isotopic material in tissues after the injection of [^{35}S]-heparin is the rate at which plasma [^{35}S]-sulphate, resulting from the desulphation of [^{35}S]-heparin, is taken up by tissues and incorporated into their sulphomucopolysaccharide. It is clear from Table 2 that resynthesis of sulphomucopolysaccharide from sulphate contributes to the presence of [^{35}S]-sulphomucopolysaccharide in tissues. This process can give a spurious indication of the disposition of [^{35}S]-heparin. The localization of radioactivity in mast cells after the injection of [^{35}S]-heparin (Loomis, 1961) is, for example, probably attributable to uptake of [^{35}S]-sulphate resulting from catabolism of heparin rather than from uptake of heparin itself, for heparin is not taken up by (neoplastic) mast cells (Green & Day, 1960).

It may be significant that the relative rates at which some tissues (notably large intestine, fat, kidney and liver) degrade exogenous [^{35}S]-heparin (Table 1) parallel the rates at which they turn over endogenous [^{35}S]-sulphomucopolysaccharide. Thus, in the rat the half-lives of endogenous sulphomucopolysaccharide in large intestine, liver, fat and kidney are 12, 15, 22 and 40 hr respectively (Robinson & Green, 1962); in catabolizing exogenous heparin (Table 1), these tissues showed the same relative order of activity. This parallel raises the interesting possibility that in some tissues the cells that catabolize exogenous heparin may be the same as those that catabolize endogenous sulphomucopolysaccharide. These cells may be fibroblasts, cells able to sequester mucopolysaccharides (Higginbotham, 1959).

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