pressed 45 days after the fractures were produced. Actually, bony union of the fractured femoral shafts had not occurred in many cases by this time. Skin-excision-stress did not result in depression of the bursting pressure of laparotomy wounds if the skin excisions were made 15 days or more before laparotomy.

Two possibilities suggest themselves to explain the stress-induced inhibition of healing. First, there may be competition between the repair process in the area of trauma (fractures or skin wounds) and the healing laparotomy wound for certain, as yet undetermined, metabolites which are essential to the healing process. Second, the induced stress may result in sufficient stimulation of pituitary-adrenal activity so that the increased endogenous secretion of adrenal cortical hormone results in inhibition of the rate of healing. The presence of hypertrophied adrenals following severe stress would favor the second hypothesis.

Summary. 1. A variety of situations, calculated to provide a chronic "stress" stimulus, have been studied in order to determine the effect of stress upon the bursting pressure of standard laparotomy wounds in rats. 2. With the exception of mild stress (skin incisions, single turpentine injection), in each case a depression of the bursting pressure of fifth day laparotomy wounds was observed. This was accompanied by increases in the weight of the adrenal glands at autopsy. 3. The duration of this period of stress-induced depression of healing appeared to vary with the magnitude and the nature of the stress stimulus.

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Hafnium Complexes for Biological Investigation.* (20496)

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A hafnium sodium mandelate complex has been used in a recent study of the distribution and excretion of hafnium in the rat(1). Hf^{181} is one of the relatively small number of readily available isotopes which have a half-life at all suitable for internal irradiation by deposition in the tissues(2). It therefore seemed important to learn whether it is practical to obtain substantially different distribution patterns using other complexing agents. Other workers have shown that the distribution patterns of colloids can be altered by variation in particle size(3,4) and that the excretion pattern of lanthanum can be changed by the use of chelating agents(5). Another reason for interest in other hafnium complexes is the fact that the mandelate preparations are often visibly cloudy at pH 7-8, while the other complexes described subsequently are more

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satisfactory in that they are more consistently clear.

Sodium catechol-3,5-disulfonate is the agent used to prepare the well known antimonial drug, fuadin. This complexing agent has been found to prevent formation of more than a trace of visible precipitate at pH 7-8 when as little as one mole was used for each 2.5 g atoms of hafnium. A hafnium oxychloride solution containing approximately 9 mg Hf/ml was prepared from irradiated hafnium oxide. A 10 ml portion of this solution was mixed with 0.492 g sodium catechol disulfonate, sodium hydroxide was added until the pH was approximately 7, and the clear solution was diluted to 25 ml. Ratio: 3 moles sodium catechol disulfonate to 1 g atom hafnium.

Sodium citrate, used in the ratio of 2 moles per g atom of hafnium, produced a solution which remained clear at pH 7 but began to become cloudy about pH 8. A solution containing Hf^{181} was prepared by adding 10 ml of the hafnium oxychloride solution to a solution of 0.252 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ in 3 ml of water, adding sodium hydroxide to a pH of 7, then diluting to 25 ml. The resulting solution appeared clear. Ratio: 1.7 moles sodium citrate to 1 g atom of hafnium.

Sodium gluconate formed a clear preparation containing as little as 1.63 moles of sodium gluconate per g atom of hafnium, but an 8:1 ratio was chosen for preparing a complex of Hf^{181}. To 2.0 ml of freshly prepared 2 M sodium gluconate solution were added 10 ml of the hafnium oxychloride solution, then sodium hydroxide to pH 7, and water to a final volume of 25 ml.

For comparison a hafnium mandelate complex was prepared from the same batch of hafnium oxychloride solution used for the preceding preparations, by adding 10 ml of the solution to 0.550 g mandelic acid, adding sodium hydroxide and water to pH 7, and diluting to 25 ml.

TABLE I. Distribution of Hf^{181} in Animals Which Received Hafnium Sodium Catechol Disulfonate Complex.

Rat No.	Specimen	Counts/min./g
1	Portion of left lobe of liver	5.3×10^3
1	" " right " " "	4.0×10^3
1	Portion at junction of lobes of liver	13.3×10^3
2	Portion of left lobe of liver	8.3×10^3
2	" " right " " "	4.6×10^3
2	Portion at junction of lobes of liver	11.6×10^3

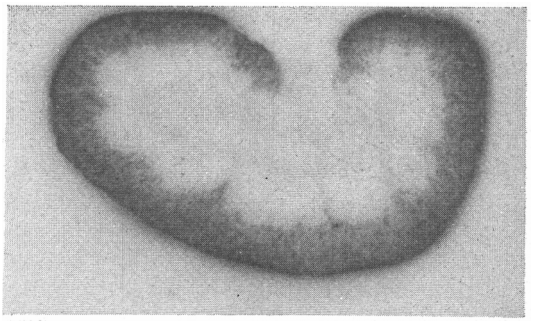

FIG. 1. Autoradiogram of kidney of dog showing uneven distribution of hafnium^{181}.

White, male, Wistar-stock rats were used for distribution experiments. A hypodermic needle attached by a short rubber tube to a syringe containing normal saline solution was inserted into the tail vein of each rat under ether anesthesia. Through another needle, 0.5 ml of the radioactive complex was injected into the rubber tube and washed into the blood stream with a small amount of the saline solution. Care was taken that the total volume injected should not exceed 2.5 ml. Since the total amount of radioactive material in each injection was less than 100 μc, it was not believed that radiation affected the distribution pattern significantly. After 96 hours each animal was lightly anesthetized by intraperitoneal injection of nembutal, a blood sample was withdrawn, and the animal was perfused with normal saline solution. The animals were dissected and the weighed organs were dissolved by heating with concentrated nitric acid. The solution was made up to a known volume, was shaken vigorously to disperse the undissolved fatty material, and a 10 ml aliquot portion was placed in a petri dish. The radioactivity was then measured by the use of a thin mica window Geiger-Mueller tube in a lead walled, vertical counting chamber.

In order to minimize the difficulties caused by undissolved fatty material, the entire solution was diluted to only 10 ml and taken for counting whenever possible. The liver was

TABLE II. Hf^{181} Distribution Ratios* 96 Hr after Injection.

Complex	No. of animals	Liver/femur Range	Liver/femur Median	Kidney/femur Range	Kidney/femur Median	Spleen/femur Range	Spleen/femur Median	Heart/femur Range	Heart/femur Median
Catechol disulfonate	12	.83 - 5.2	1.9	1.3 -13.4	6.2	.78 - 3.7	2.05	.28 -.98	.53
Gluconate	14	.042- .30	.14	.15- 2.3	.79	.077- .70	.18	.018-.11	.066
Citrate	8	.20 - 1.21	.53	.29- 2.1	.52	.016- .40	.14	.023-.10	.051
Mandelate	12	1.6 -18	5.8	.69- 2.1	1.35	2.7 -12.1	5.7	.04 -.9	.32

* Based on counts/g of tissue.

usually minced into very small pieces and a representative sample was taken for digestion and counting. It was not found satisfactory to digest a single strip of the liver as a sample because of the variations in uptake within a single organ as illustrated by the examples shown in Table I. The uneven distribution of Hf^{181} in another organ is illustrated in Fig. 1.

Rather wide variations were observed within the distribution patterns for each of these complexes, but in spite of this fact some striking differences between complexes were apparent. Several of these are illustrated in Table II summarizing distribution ratios at 4 days after injection. The gluconate and citrate complexes behaved very much alike, but the lowest liver/femur ratio in the catechol disulfonate series was nearly 3 times the highest liver/femur ratio in the gluconate series. The liver/femur ratio was even higher in the mandelate series. The kidney/femur ratio was highest in the catechol disulfonate series, while the spleen/femur ratio was highest in the mandelate series.

In all series the concentration of hafnium in the femur was larger than in the heart, but the median heart/femur ratio for the mandelate series was 0.31 while for the catechol disulfonate, citrate, and gluconate series the median figures were 0.53, 0.053, and 0.075. The uptakes in both heart and leg muscle were low, but the uptake in the heart was frequently several times as great as in the leg muscle. The median ratios were: for citrate 2.9, for gluconate 3.0, for mandelate 6.5, for catechol disulfonate 5.9. The median ratios of counts per g of adrenal gland/femur were 2.1 in the mandelate series, and 1.2 in the catechol disulfonate series.

A total of 45 additional rats were used in the study of the distribution patterns for the 3 new complexes at periods from one hour to 16 days after injection. While the data suggest that some gradual change in distribution ratios may occur with time, the evidence is not conclusive. The median ratios shown in Table II would have been little different if the data for the other periods had been included. There was, however, evidence that the concentration of hafnium in the blood decreased rapidly and that excretion of hafnium in the urine occurred much more rapidly during the first few hours than later. A number of the experimental animals were placed in metabolism cages immediately after injection and a conventional apparatus was used to collect urine and feces separately. The activity found in the feces was low. Of 5 rats which received the mandelate complex, none excreted over 8% of the injected dose in the urine within 24 hours, while 9 of 12 rats which received the gluconate and 7 of 8 rats which received the catechol disulfonate excreted half or more of the injected hafnium within 4 hours.

The marked differences in the distribution patterns of hafnium administered with different complexing agents suggest the possibility that the usefulness of radioactive isotopes may be increased by directing the localization in tissues through the use of suitable complexing agents.

Summary. The distribution of Hf^{181} in rats was different when different hafnium complexes were administered. The median liver: femur concentration ratios with various complexing agents were in order mandelate >catechol disulfonate >citrate >gluconate. Differences were observed in other organs also.

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Effect of Cortisone Upon Formation of Liver Glycogen from Glycine.* (20497)

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Gluconeogenesis from protein in the fasting animal has been established as being mediated by the adrenal cortex(1-4). Hess and Shaffran(5) have studied the rate of liver glycogen formation from glycine in fasted normal rats. The present paper is concerned with the effect of cortisone upon the rate of glycogen formation from glycine in the fasted normal rat.

Experimental. White rats, weighing 100 to 150 g, fasted for 24 hours were fed, by stomach tube, 450 mg of glycine dissolved in 2.5 ml of water. Cortisone acetate in saline suspension (Cortone Merck) was given intramuscularly at the same time as the glycine at 2 dosage levels, 2.5 and 5.0 mg. Controls receiving only cortisone were also studied. At the end of the experimental periods the animals were sacrificed and liver glycogen determined by the method of Good, Kramer, and Somogyi(6). The animals were kept in metabolism cages during the fasting and test periods and urine was collected for total nitrogen determination by the micro-Kjeldahl method. The results are given in Tables I, II, and III; each value is the average of the results from 4 to 6 animals. Duplicate determinations were made on each sample. For comparison the data for the formation of glycogen from glycine alone(5) are included.

Cortisone alone produced the increase in liver glycogen observed by many other investigators. The 5 mg dose induced the maximum deposition at 40 hours, the period from 31 to 48 hours giving a fairly uniform value. A peak was found at 16 hours followed by a slight decline and a subsequent marked increase; a similar effect was noted when cortisone plus glycine were given. The 2.5 mg dose produced a maximum at 24 hours, the amounts found at 31 and 48 hours were slightly less. The maximum amount of glycogen formed following the 5 mg dose of cortisone was almost twice that produced by feeding glycine alone and the maximum formed following the 2.5 mg dose was just slightly less than the peak for glycine alone. However, the maximum glycogen value following glycine was reached earlier.

When 5 mg of cortisone was given at the

TABLE I. Concentration of Liver Glycogen Following Glycine, 5 mg Cortisone, and Glycine plus Cortisone.

	1	2	3*	4
Time, hr	Glycine	Cortisone	Cortisone + glycine	Change
	%			
2	.25	.23	.025	—.455(<.01)†
4	.95	1.80	2.50	—.25 (<.10)
6	1.35	1.60	2.60	—.35 (<.01)
12	2.40	1.80	4.60	+.40 (<.01)
14	2.50	2.35	5.15	+.30
16	2.55	2.40	5.20	+.25
24	1.80	1.90	3.70	.0
31	.40	3.80	4.10	—.10
40	.20	4.00	4.40	+.20
48	.20	3.70	3.90	.0

* Column 3—(Column 1 + column 2).

† P values for the change based upon the calculation for t. From 14 hr to 48 hr the changes are not significant.

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