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PROGRESS REPORT ON THE METABOLISM OF POSTUM

III. Tracer Studies in Two Human Subjects

Prepared by

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November 11, 1944

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PROGRESS REPORT ON THE METABOLISM OF POSTUM

III. Tracer Studies in Two Human Subjects

Summary.

The assimilation and excretion of sublethal doses of postum chloride by the human body has been studied in two human subjects. The excretion studies were carried out chiefly on an adult male in good general health except for generalized lymphosarcoma. The study of the distribution of postum in human tissues was carried out on a subject having acute lymphatic leukemia, where an autopsy was performed six days after injection of the material. Dosages were respectively 0.30 and 0.17 microcuries per kilogram of body weight, administered by intravenous injection.

In both subjects, the daily excretion of postum in the urine followed an irregular concave descending curve. In the first, more nearly normal subject, the urinary excretion was approximately 0.2% of the dose on the first day, 0.05% per day on the second and third days and 0.03% per day for the remainder of the 8-day period of observation. A sample obtained 43 days after the injection indicated a daily urinary excretion of 0.02% of the dose at that time. The urinary excretion of the second subject started at 0.8% of dose the first day and leveled off at about 0.1% per day for the remainder of the six-day period.

The daily excretion of postum through the feces of the first subject increased approximately linearly from 0.44% of dose on the first day to 4.2% on the sixth day after injection, and then dropped to 0.92% on the seventh day. Only one fecal sample from the leukemic patient was analyzed. It contained approximately the same percentage of the dose per gram of fecal material as was found in the corresponding sample from the first patient. All of the values for urinary and fecal excretion fell within the range found in rats except for the first urine sample from the leukemic patient.

Periodic sampling of blood from these subjects indicated that the concentration of postum therein remained fairly constant during the original sampling period. It fluctuated around 0.004 per cent of the dose per ml. of whole blood in the first subject and 0.002 per cent in the leukemic subject. Five weeks later the value in the first subject was 0.0006% dose per ml.

Analysis of tissues secured through an autopsy on the second subject revealed that the bile fluid contained the greatest concentration of postum, 0.091 per cent of dose per gram. This was followed in order by the concentrations in the liver, heart, spleen and kidneys, which ranged from 0.021 to 0.014 per cent of dose per gram. Concentrations in all other tissues were below 0.005 per cent of dose per gram. Most of the tissue data on this human subject are quite similar to the corresponding data on rats, and it seems that the few marked differences that were found may have been due in some measure to abnormalities in the patient rather than to inherent differences between humans and rats with respect to the assimilation of postum.

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Detailed Report of Experimental Procedure and Results.

Case No. 1.

On September 11, 1944, a total of 22.6 microcuries (0.3 microcuries per kilogram of body weight) of postum chloride were administered intravenously to [redacted], a 35-year old male, being treated for generalized lymphosarcoma. The postum was administered in 10 ml. of sterilized isotonic saline solution at pH 7.6. In order to determine as accurately as possible the amount actually administered, a second 10 ml. sample of the solution was then immediately drawn into the syringe used for the injection, and expelled into an acid solution at approximately the rate used for the actual injection. The acid solution was set aside for subsequent radioactivity measurement. This "dummy injection" procedure has been followed since it was discovered that under some conditions as much as one-quarter of the postum in a solution may plate out on the needle during injection. In the case of human experiments there is involved a further loss of one-quarter to one-half of the postum during sterilization of the neutral solution in the autoclave, and this factor is also controlled by the "dummy injection."

In determining the amounts excreted in the daily urine collections, the total daily volume was measured and a convenient aliquot (about five per cent of the total) taken, acidified to half molar with concentrated hydrochloric acid and plated directly onto a silver-foil disc, which was read in the ionization chamber. The daily urinary excretion of postum by the first subject, and the concentrations of postum per ml. of urine are given in Table I and shown graphically in Fig. 1. Due to an oversight, the use of a glass urinal for these collections was not specified, so that it is possible that this ambulatory patient used a stainless steel urinal, and probable that the second, bedridden patient used the metal vessel. A subsequent check indicated that use of the metal vessel would result in the loss of less than one-tenth of the postum in the specimen.

Fecal samples were obtained daily from this subject for seven days after injection. Each sample was weighed and a suitable known volume of three molar hydrochloric acid added to facilitate proper mixing and sampling. Aliquots of each day's sample were digested by the previously described procedure involving a perchloric acid-nitric acid mixture. The resulting solutions, free from organic matter, were neutralized with sodium hydroxide and acidified to half molar H_3O^+ with concentrated hydrochloric acid. The postum was plated from these solutions onto silver-foil discs and measured in an ionization chamber, as outlined in a previous report. The total daily excretion through the feces and the concentrations of postum therein are given in Table I and shown graphically in Fig. II. The concentrations of postum in the blood of this first subject are also shown in Table I.

Case No. 2.

On September 13, 1944, a total of 11.8 microcuries (0.17 microcuries per kilogram of body weight) of postum chloride were administered by intravenous injection to [redacted], a 32-year-old male. This second subject had acute lymphatic leukemia, which was the cause of his death six days

[redacted]

after injection of the material. As in the case of the first subject, the dose administered was determined by means of a "dummy injection" through the same needle immediately after the actual injection.

The total daily excretion and the concentration of postum in the urine are shown in Table II and Fig. III. The urine samples showed some red blood cells on microscopic examination, and the first day's sample in particular was very dark colored. As both red cells and bile contain very much higher concentrations of postum than does urine, the presence of small amounts of either might raise the urine concentration beyond the range for normal subjects.

Quantitative collection of the feces was not carried out on this patient, but a single sample obtained one day after the injection contained 0.008% of the dose per gram of fecal matter, a value similar to that found for the corresponding sample from the first patient.

The concentration of postum in the blood of [redacted] is also shown in Table II. An autopsy was performed and tissue samples taken about an hour after the death of this patient. The tissue samples were digested, plated out on silver foil, and their radioactivity measured according to the methods described in previous reports. The concentrations of postum found in the various tissues, expressed as the percentage of the total dose per gram of tissue, are tabulated in Table III. The bile obtained from the gall-bladder showed the highest concentration, being more than four times as high as the liver, heart, spleen and kidney concentrations, seventy times the concentration in the blood, and fifteen hundred times the concentration in the urine.

Table IV gives the percentage of the dose found in various whole organs or tissues of the human subject, and for comparison shows the corresponding average values for five rats (four sacrificed 10 days, and one 20 days, after injection of postum). A better method of comparison, since it avoids uncertainties as to the total weight of a given organ or tissue, is shown in Table V. This shows the ratio of the amount of postum found per gram of <u>tissue</u> to the amount of postum administered per gram of <u>subject</u>. If a dose of injected material were evenly distributed throughout the body, and excretion were negligible, then the ratio would be unity for all tissues. Conversely, a value over unity indicates that the organ takes up postum to a greater extent than is accounted for by simple uniform distribution. The data are compared with corresponding values from the five rats mentioned above.

Discussion.

An examination of Tables IV and V shows that, although for the most part the distribution of postum in the human and in the rat is similar, there are a few wide deviations. The heart, liver, skin, muscle, plasma and testes show higher concentrations of postum in the human than in the rat (when put on a comparable basis as in Table V), while the lymph nodes, bone marrow and blood show lower concentrations in the human. The picture is approximately the same on the basis of the amount taken up by the whole tissue (Table IV), except for differences due to

the relative sizes of certain organs in rat and man. For example, the human testes and allied genital structures contain a lower percentage of the dose than do those in the rat, due to the proportionately smaller size of these organs in the human.

The values shown in Table IV indicate a recovery from the human tissues of more postum than was originally administered. The inconsistency probably arose mostly from the fact that adequate sampling procedures could not be carried out, and a single small tissue sample had to be taken as representative of a whole organ or tissue. There was, in addition, the possibility of contamination of the tissue samples during preparation for measurement, and the exceptionally high value obtained for heart muscle may have been due to this cause. In the rat, heart muscle ordinarily had a postum concentration similar to skeletal muscle, providing care was taken to remove excess blood from the heart. Accordingly, when a high value was obtained from the sample of human heart, a second aliquot from the heart digest was plated out and found to give a reading 30 per cent higher than the first. This increase of activity on standing indicated that, in spite of what were thought to be adequate measures for decontamination and segregation of glassware, the heart digest was probably stored in a contaminated bottle. Rather extreme measures have since been instituted for avoiding further occurrences of this type.

It is obviously impossible to evaluate accurately the effect of the pathological state of the human subject upon the distribution of postum in his body, but there are some points that seem worthy of mention. The possible effect of the kidney damage on the values for urinary excretion has already been discussed. The principal autopsy findings were many petechial hemorrhages, spleen and bone marrow "crammed" with round cells, hyperplasia of the young lymphocytes in the lymph nodes, and confirmation of the jaundice and anemia noted earlier. It seems possible that the infiltration of round cells might have lowered the spleen and bone-marrow values from what they would have been in a normal subject, though that (like the hyperplasia in the lymph nodes) might conceivably work either way. Anemia would be expected to reduce the postum concentration in blood because of the relatively high concentration ordinarily found in red blood cells. The jaundice, though not of great interest to the pathologists in this case, is perhaps of more interest in this tracer experiment, due to the very high concentration of postum in the bile. During jaundice, the concentration of bile is reported to become particularly high in the liver, skin, muscle and plasma. Since these are tissues in which the human had relative postum concentrations considerably higher than the rats, it seems possible that the jaundice contributed in some measure to the high values.

Thus, as the data stand, they indicate a fairly close similarity between the human and the rat with respect to the metabolism and distribution of postum, and it seems probable that a man in good general health would show a closer similarity.

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TABLE I.

Percentage of dose in urine, feces and blood of [redacted] (generalized lymphosarcoma) after intravenous administration of 0.3 microcuries postum chloride per kilogram of body weight.

Days after injection	% Dose excreted in Urine	Feces	Percentage of dose per gram in Urine	Feces	Whole Blood	Plasma	Blood cells
1	0.203	0.44	.000125	.0048	.0037	.0016	.0070
2	.048	1.35	.000052	.0161	---	---	---
3	.056	1.37	.000038	.0240	.0047	.0014	.0080
4	.038	1.92	.000024	.0194	---	---	---
5	.031	3.55	.000025	.0295	.0045	.0013	.0073
6	.030	4.16	.000033	.0359	---	---	---
7	.021	0.92	.000019	.0144	.0035	.0007	.0073
8	.033	---	.000024	---	---	---	---
43	.016	---	.000020	---	.0006	.0004	.0009

TABLE II.

Percentage of dose in urine, feces and blood of [redacted] (acute lymphatic leukemia) after intravenous administration of 0.17 microcuries of postum chloride per kilogram of body weight.

Days after injection	% Dose excreted in Urine	Percentage of dose per gram of Urine	Whole Blood	Plasma	Blood Cells
1	.836	.000584	.0023	.0016	.0055
2	.137	.000156	---	---	---
3	.190	.000105	.0025	.0021	.0046
4	.114	.000079	---	---	---
5	.107	.000036	.0017	.0012	---
6	.044	.000031	.0013	.0009	.0024

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TABLE XII.

Concentrations of postum in tissues of [redacted] (acute lymphatic leukemia) six days after intravenous administration of 0.17 microcuries postum per kilogram of body weight.

Tissue	% Dose per gram
Bile	.0110
Liver	.0110
Heart	.0100
Spleen	.0170
Kidney	.0186
Testes	.0043
Lung	.0041
Skin (from chest)	.0039
Omental lymph nodes	.0033
Sternal marrow	.0031
Vertebral marrow	.0028
Pancreas	.0027
Cervical lymph nodes	.0026
Submaxillary glands	.0026
Blood cells	.0024
Muscle (from chest)	.0017
Bone (rib)	.0017
Thymus	.0015
Duodenum	.0015
Whole blood	.0013
Epididymis	.0011
Stomach	.0009
Blood plasma	.0009
Seminal vesicles	.0009
Thyroid	.0008
Aorta	.0003
Prostate	.0007
Rectum	.0001

[redacted]

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TABLE II.

Percentages of dose in entire tissues of [redacted] (acute lymphatic leukemia) six days after intravenous administration of 0.17 microcuries of postum chloride per kilogram of body weight, with same data for 3 rats.

Tissue	[redacted]	Rat Average	Rat Range
Muscle[1]	[illegible]	[illegible]	[illegible]—17.10
Skin[2]	[illegible]	[illegible]	[illegible] 14.53
Liver	[illegible]	[illegible]	[illegible] 15.35
Skeleton[3]	[illegible]	[illegible]	1.17 11.25
Whole Blood[4]	4.10	[illegible]	3.08 6.32
Kidneys	[illegible]	[illegible]	[illegible] 7.44
Blood cells[5]	[illegible]	[illegible]	[illegible] 5.24
Spleen	[illegible]	[illegible]	1.10 3.57
Lungs	[illegible]	1.07	0.68 1.29
Testes	[illegible]	[illegible]	0.45 0.56
Plasma[6]	[illegible]	[illegible]	0.19 0.48
Stomach	[illegible]	[illegible]	0.29 0.46
Heart	[illegible]	[illegible]	0.04 0.37
Pancreas	[illegible]	[illegible]	0.15 0.37
Seminal vesicles	0.02	[illegible]	0.13 0.18
Brain	—	[illegible]	0.04 0.11
Prostate	0.03	0.07	0.03 0.10
Adrenals	—	[illegible]	0.03 0.05
Thyroid	0.03	0.01	— —
	169.23	66.5%	

1. Total weight of muscle tissues estimated as 41.7% of body weight in man and 45.40% of body weight in rat. Muscle from chest in man, from leg and/or abdomen in rats.

2. Total weight of skin estimated as 18% of body weight in man and rats. Skin from chest in man, from abdomen (and including fur) in rats.

3. Weight of total skeleton estimated as 15.9% of body weight in man and 10.9% in rats. Bone sample from rib in man, from femur or tibia in rats.

4. Total weight of blood estimated as 4.0% of body weight in man and 3.5% in rats.

5. Derived by difference between plasma and whole blood.

6. Total weight of plasma derived from application of hematocrit values to estimated total blood weight.

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TABLE V.

Amounts of postum found per gram tissue in units of amount administered per gram total body weight. Values for [redacted] compared with corresponding values and ranges for five rats, four of which were sacrificed at 10 days and one at 20 days after injection of postum. (Values greater than 1.0 indicate a concentration of postum higher than would be accounted for by uniform distribution of the postum administered.)

Tissue	[redacted]	Rat Average	Rat Range	
Spleen	11.01	11.[illegible]	4.[illegible]8	23.[illegible]
Kidneys	8.67	7.35	4.70	12.[illegible]
Cervical nodes	1.67	6.03	2.24	10.[illegible]
Bone marrow[1]	1.9[illegible]	5.28	0.[illegible]5	7.[illegible]
Omental nodes	2.41	[illegible]	[illegible]	6.[illegible]
Pancreas	1.7[illegible]	[illegible]	0.[illegible]	4.[illegible]
Liver	15.70	[illegible]	1.24	4.[illegible]
Blood cells	1.54	2.31	2.[illegible]	2.[illegible]5
Whole blood	0.85	[illegible]	1.13	[illegible]
Thymus	0.99	1.38	1.[illegible]7	[illegible].29
Lungs	2.65	1.65	1.[illegible]2	2.51
Submaxillary glands	1.64	1.19	0.96	1.43
Heart	13.12	1.12	0.16	2.36
Prostate	0.45	0.98	0.47	1.[illegible]
Skin[2]	2.4[illegible]	0.58	0.38	0.30
Bone[3]	1.0[illegible]	0.56	0.05	1.03
Testes	2.73	0.51	0.44	0.33
Seminal vesicles	0.63	0.46	0.57	0.63
Muscle[4]	1.09	0.29	0.17	0.38
Stomach	0.58	0.28	0.20	0.42
Blood plasma	0.57	0.14	0.03	0.19

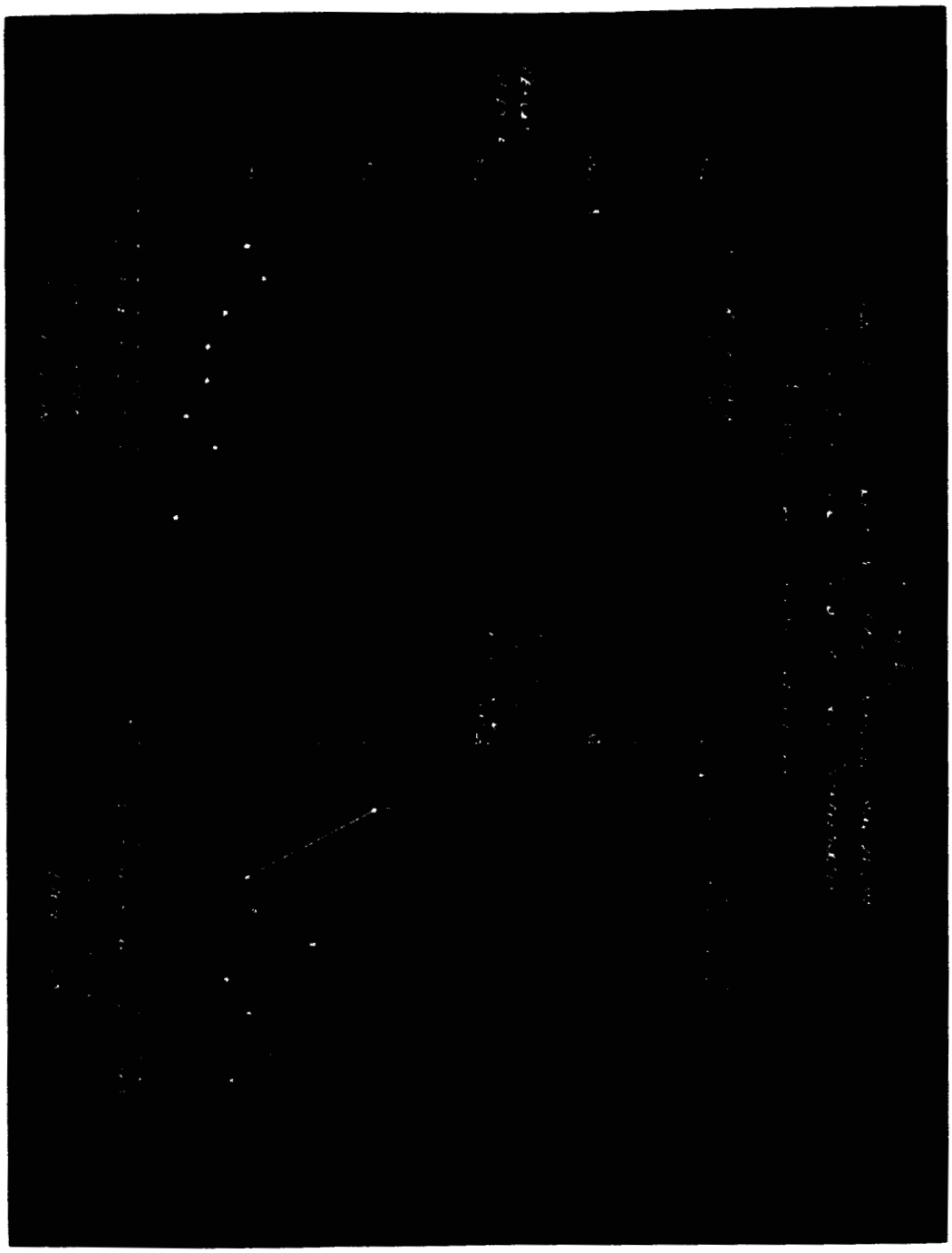
1. Bone marrow sample taken from sternum and vertebrae in human (no significant difference between the two) and from femur or tibia in rats.

2. Skin sample from chest in human; from abdomen (and including fur) in rats.

3. Bone sample from rib in man; from leg in rats.

4. Muscle from chest in man; from leg and/or abdomen in rats.

[redacted]



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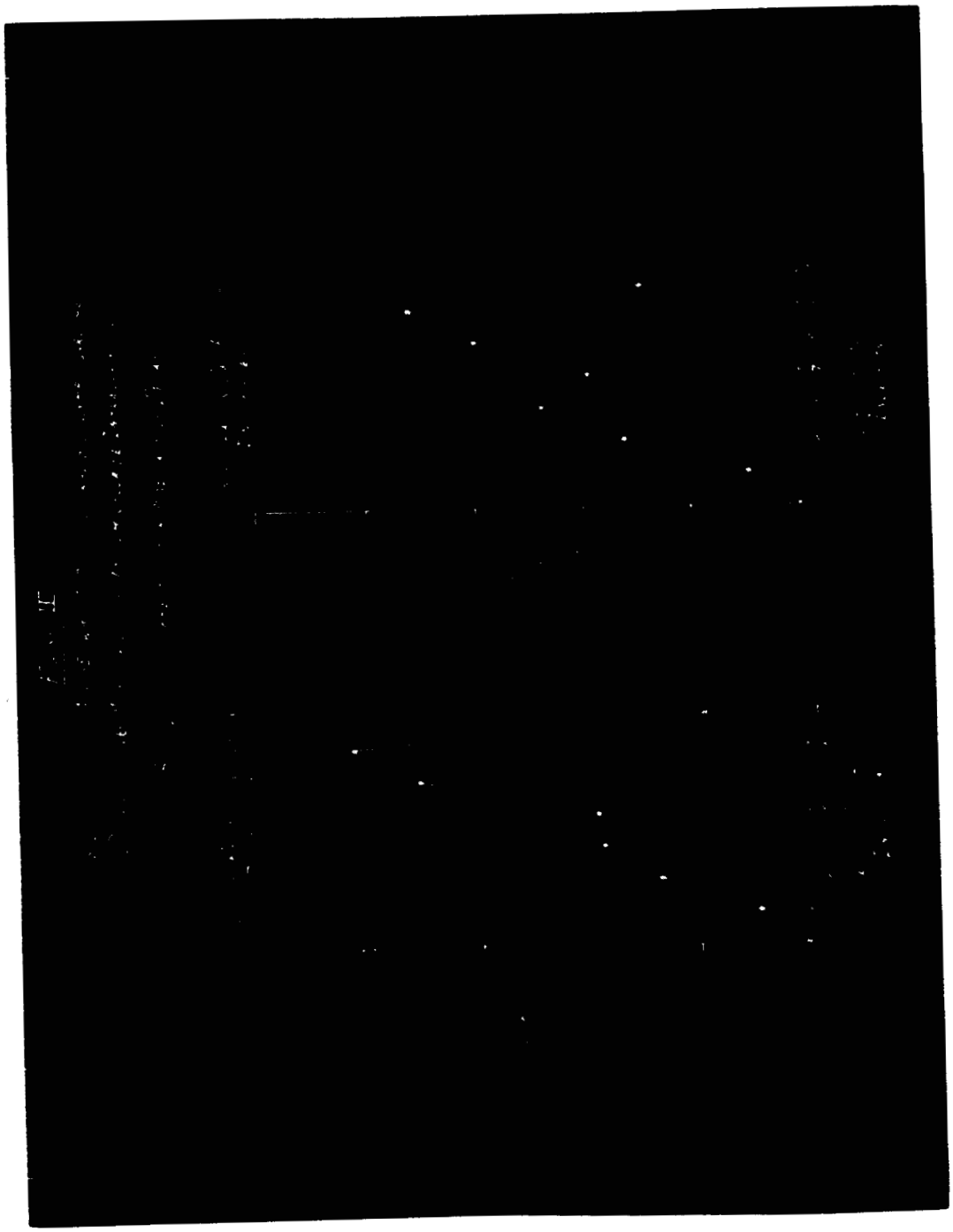

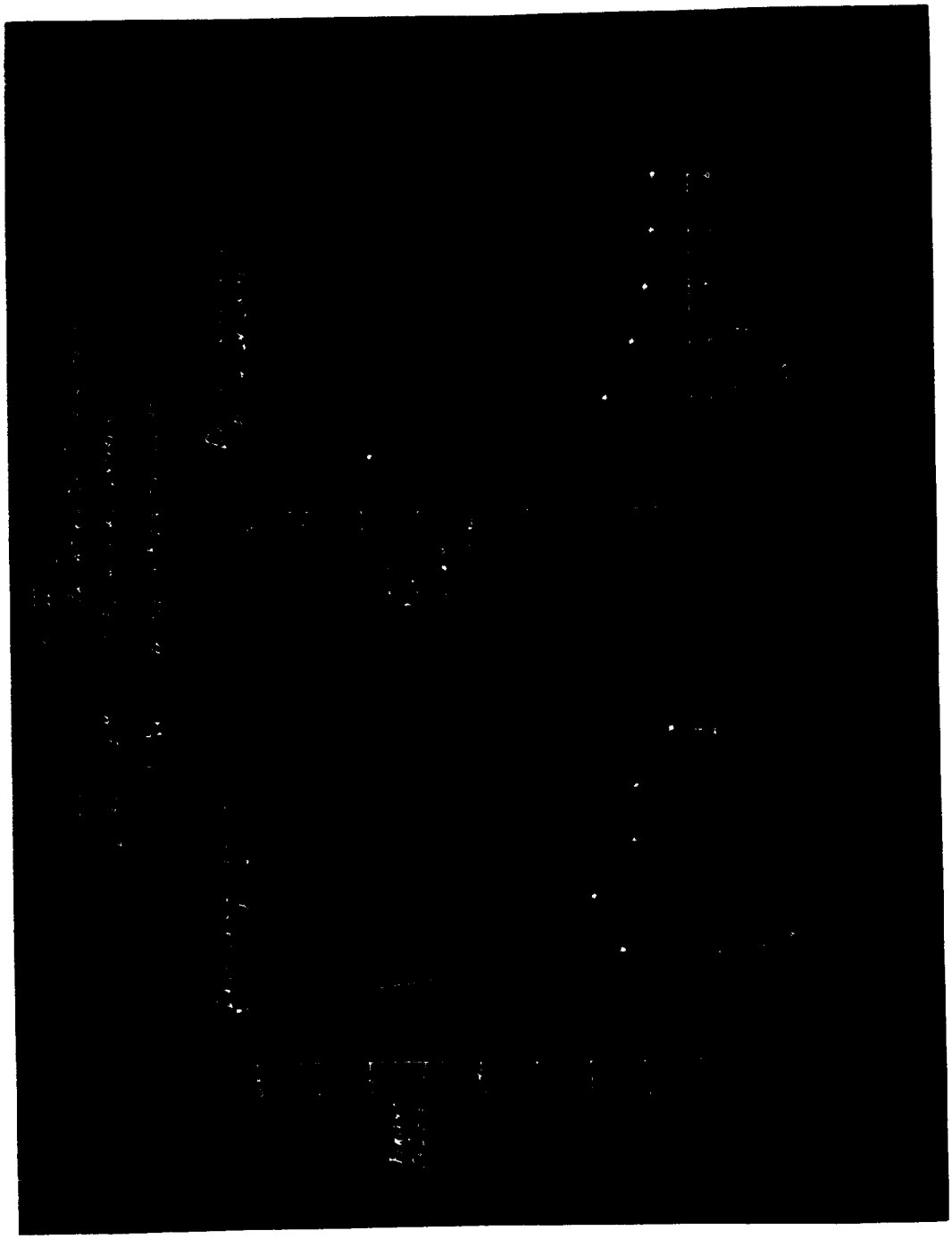

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