Rate of Incorporation of C^{14}-Glycine Injected into Adult Female Frogs[1]

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In a previous investigation on uptake of radioactive glycine in frogs, it was reported (Kemp, '55, '56) that "conspicuously high relative uptake of glycine was detected in mucosal epithelium of stomach and intestine, in epidermis and in peripheral cytoplasm of oocytes synthesizing yolk." Evidence was presented then that sites of autoradiographic localization of glycine may correspond to sites of protein synthesis, although it was recognized (Kemp, '56) that glycine may be utilized in a variety of metabolic pathways. At about the same time, Ficq ('55) demonstrated that radioactivity of sections of ovaries from frogs injected with glycine-1-C^{14} could be reduced by treating sections with ribonuclease. Residual radioactivity after exposure to ribonuclease was approximately the same as that which could be detected autoradiographically after injecting frogs with dl-3-phenylalanine-3-C^{14}. Ficq surmised that the radioactivity removed by ribonuclease represented glycine incorporated into RNA, while the residual activity was probably due to protein-bound glycine. She and her collaborators, plus a number of other workers (Ficq and Errera, '55, '58; Ficq and Brachet, '56; Sirlin and Brahma, '59; Waddington and Sirlin, '59) have since employed C^{14}-labeled phenylalanine as a fairly specific precursor for detecting sites of protein synthesis. Another group of workers (Sirlin and Waddington, '56; Leblond, Everett and Simmons, '57; Giudice and Monroy, '58; Céas and Naselli, '58; Bodemer and Everett, '59; Greenwald and Everett, '59) have utilized S^{35}-labeled methionine as a specific precursor for protein synthesis.

Although glycine may be converted to serine or used for synthesis of carbohydrates, glutathione, purines and nucleic acids (Henriques, Henriques and Neuberger, '55; Ficq and Brachet, '56; Zamecnik, Keller, Littlefield, Hoagland and Loftfield, '56; Leblond, Everett and Simmons, '57), and possibly other compounds in addition to protein, part of an injected quantity of glycine is either directly or indirectly incorporated into protein (Hayashi and Herrmann, '59; Herrmann and Schultz, '58; Herrmann, Lerman and White, '58; Schultz and Herrmann, '58).

The aim of the work reported in this paper was to demonstrate more precisely than in earlier investigations (Kemp, *op. cit.*) the sites and rates of incorporation of radioactive glycine in selected organs of the adult female frog, namely, skin, organs of the digestive system, ovary, kidney and spleen.

MATERIALS AND METHODS

Fifty-three adult females of Rana pipiens purchased from a supplier in Wisconsin during the summers of 1959 and 1960 were kept for several days at room temperature in about an inch of water in gallon jars equipped with drilled bakelite covers. During this period of maintenance, animals were force-fed with liver every other day to insure that they would be in a good nutritional state when used for an experiment. Just before injection of a group of animals, water was poured out of the jars and replaced with a folded piece of wet paper toweling. An animal to be injected was grasped with the gloved right hand inside the jar, then removed and transferred to the gloved left hand holding a dry paper towel. Held inside the towel, the animal had little chance to

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move while being injected through the ventral body wall with either 5 μc or 10 μc of C^{14}-glycine in 0.5 ml of distilled water. Neither carbon atom of the glycine was specifically labeled. After injection the animal was returned to its jar and was not fed thereafter.

In the 1959 series of experiments, 10 animals injected with 5 μc of radioactive glycine were sacrificed at intervals of 4, 8, 16, 24 and 72 hours after injection. Since autoradiographic activity was pronounced in tissues of animals fixed as early as 4 hours after injection, another series of 43 animals injected with 10 μc of radioactive glycine in the summer of 1960 were sacrificed at 5, 10, 20, 30, 40, 50 and 60 minutes after injection, as well as at 2, 4, 8, 16, 24, 48, 72 and 96 hours. One animal sacrificed at each interval in the 1960 series was selected for comparing autoradiograms and specific activity of proteins in the organs studied.

Animals in the 1959 series were fixed in alcohol-acetic acid as recommended by Ficq ('59). Those of the 1960 series were fixed in Bouin's fluid. Paraffin sections were cut at 10 μ and affixed to slides with chrome-alum gelatin (Ficq, '59). Autoradiograms were prepared both with No-screen x-ray film, as previously described (Kemp, '56), and with Kodak permeable base stripping film. Kodak AR-10 stripping film and Kodak NTB-3 liquid emulsion, supplied by Eastman Kodak Co., were also used for comparison with the permeable base film. X-ray film was routinely exposed to tissue sections in a black plastic slide box kept in a dessicator in a refrigerator at 4°C for 4 days. Film was developed with Kodak x-ray film developer for 5 minutes. Stripping film or coated emulsion were exposed similarly for 20 or 21 days and developed two minutes with Kodak developer D-19b.

Slides used for making autoradiograms on x-ray film were stained with hematoxylin and eosin. Slides with attached autoradiograms on stripping film or coated emulsion were stained with methyl green and pyronin (Taft, '51). Photomicrographs were taken on Kodak Wratten M plates with a Spencer photomicrographic camera.

Protein samples for Geiger counting were prepared by the procedure previously described (Kemp, '56). Samples were plated on aluminum discs and counted with a Nuclear-Chicago scaler and Geiger-Müller tube with a thin end-window. For determination of specific activity, weights of the samples were measured after they had been dried for two hours in an oven at 105°C.

RESULTS

Speed of incorporation of glycine

The relative speed of incorporation of glycine-C^{14} as detected by autoradiograms on x-ray film is recorded in table 1. Evaluation of the autoradiograms is subjective but does permit rough comparisons of radioactivity. Table 1 shows first that sections of ventral skin, liver and kidney from an animal killed 5 minutes after injection of 10 μc of C^{14}-glycine had detectable (+) radioactivity. By 10 minutes low radioactivity could also be demonstrated in dorsal skin, ovary, duodenum and pancreas, lower small intestine and spleen. There was no detectable radiactivity in either stomach or ovary of the specimen fixed at 20 minutes after injection, but by 30 minutes all organs showed some activity. Skin, duodenum and pancreas, liver and kidney were moderately radioactive (++) at 30 minutes and maintained this level up to 50 minutes. Kidney dropped back with the organs having low activity (ovary, stomach, small intestine and spleen) at one hour and two hours. At 4 hours, however, only ovary and stomach remained at low activity. Skin and liver were strongly (+++) active by this time. Duodenum and pancreas, plus spleen, advanced to strong radioactivity at 8 hours. Very strong (++++) radioactivity was first attained by ventral skin and kidney at 16 hours. Dorsal skin, ovary, duodenum and pancreas, small intestine and spleen reached very strong activity at 24 hours. Activities in ovary, stomach, and duodenum had declined somewhat in the 48-hour specimen but were either strong or very strong in all organs at 72 hours. Radioactivities of most organs were lower in the 96-hour specimen than in the one fixed at 72 hours. Scrutiny of tables 1 and 2 reveals a close correspondence between the estimated activities of sections and the specific activities of isolated proteins from the same organs.

TABLE 1

Rate of incorporation of C^{14}-glycine into various organs as detected autoradiographically on No-screen x-ray film exposed 4 days at 4°C and developed 5 minutes

Time from injection to sacrifice	Relative radioactivity detected in sections of ventral skin (vs), dorsal skin (ds), ovary (ov), stomach (st), duodenum and pancreas (dp), small intestine (si), liver (li), kidney (ki), spleen (sp). +, low activity; ++, moderate activity; +++, strong activity; ++++, very strong activity
5 minutes	Negative in ds, ov, st, dp, si, sp; + in vs, li, ki.
10 minutes	Negative in st; + in vs, ds, ov, dp, si, li, ki, sp.
20 minutes	Negative in ov and st; + in vs, ds, dp, si, li, ki, sp.
30 minutes	+ in ov, st, si, sp; + + in vs, ds, dp, li, ki.
40 minutes	+ in ov, st, si, sp; + + in vs, ds, dp, li, ki.
50 minutes	+ in ov, st, si, sp; + + in vs, ds, dp, li, ki.
1 hour	+ in ov, st, si, ki, sp; + + in vs, ds, dp, li.
2 hours	+ in ov, st, si, ki, sp; + + in vs, ds, dp, li.
4 hours	+ in ov, st; + + in dp, si, ki, sp; + + + in vs, ds, li.
8 hours	+ in ov; + + in st, si, ki; + + + in vs, ds, dp, li, sp.
16 hours	+ + in ov, st, dp, si; + + + in ds, li, sp; + + + + in vs, ki.
24 hours	+ + + in st, li, ki; + + + + in vs, ds, ov, dp, si, sp.
48 hours	+ + in ov, st; + + + in dp, ki; + + + + in vs, ds, si, li, sp.
72 hours	+ + + in ov, st, si, li, ki; + + + + in vs, ds, dp, sp.
96 hours	+ in st; + + in dp, si, ki, sp; + + + in vs, ds, ov, li.

How radioactivity was distributed among tissue components of various organs in an animal sacrificed 24 hours after injection is shown in plate 1. One can clearly see localizations in all organs except ovary and spleen. Kidney was not tested with stripping film. In skin (figs. 1 and 2) there is clear localization of radioactivity in the epidermis. It is probable that some individual connective tissue cells of the dermis are labeled, but their density is so much less than that of epidermal cells that the autoradiogram has a higher concentration of silver grains, including beta tracks, over the epidermis.

Epithelium of stomach (fig. 3), duodenum and lower small intestine (fig. 4) has higher radioactivity than either connective tissue of lamina propria and submucosa, or smooth muscle of the muscularis mucosae or of the mucularis proper. Where cells of the lamina propria were crowded, as between crypts of intestinal glands, activity appeared to be greater than where connective tissue cells were more sparsely distributed. Exocrine cells of the pancreas (fig. 5) are highly radioactive. Cells of pancreatic ducts, connective tissue, blood vessels and islets of Langerhans are much less so. Parenchyma cells of the liver (fig. 6) have high radioactivity; bile ducts, connective tissue and blood vessels are less active. The ovaries in most of the specimens examined were immature. Oocytes, as illustrated in figure 7, were chiefly at stage Y°, prior to the onset of vitellogenesis (Kemp, '53), and showed no conspicuous localization of radioactivity. The spleen (fig. 8) had relatively low radioactivity throughout and showed no major localization, although some regions containing densely packed lymphoid cells appeared slightly more radioactive than other regions.

Radioactivity of isolated proteins

The specific activity of proteins isolated from various organs of 15 individual frogs is listed in table 2. The figures indicate relatively low incorporation of glycine into protein during the first 20 minutes after injection but a general rise starting at 30 minutes. Incorporation at 8 hours was greater in all organs than at 4 hours and was still greater at 16 hours. Relatively high incorporation was maintained up to 72 hours, although activity in the 48-hour specimen was less than in the 24-hour specimen. Activity in the 96-hour animal was considerably reduced from that at 72 hours.

One unexpected finding was that ventral skin generally had a higher specific activity than dorsal skin. Possibly the greater thickness of the dermis dorsally accounts for the difference. Activity in the stomach was relatively low in all specimens, probably because of the high proportion of connective tissue and smooth muscle in this organ. It may be also that the lining epi-

TABLE 2

Specific activity (counts/min/mg) of proteins isolated from various organs of frogs injected with 10 μc of glycine-C^{14} and sacrificed after designated intervals

Time from injection to sacrifice	Counts/min/mg of protein from various organs of individual frogs							
	Ventral skin	Dorsal skin	Ovary	Stomach	Duodenum and Pancreas	Small intestine	Liver	Kidney
5 minutes	16.4	6.5	4.2	0.1	3.0	3.0	5.7	1.5
10 minutes	16.0	0.9	7.0	0.9	16.3	4.3	19.6	20.8
20 minutes	13.3	18.7	3.8	2.0	14.5	11.9	14.9	7.5
30 minutes	40.8	12.4	11.4	2.6	43.8	26.7	42.0	31.3
40 minutes	39.5	31.9	17.9	3.3	20.0	15.0	50.0	29.7
50 minutes	59.0	24.5	10.6	5.7	30.5	16.3	53.1	30.0
1 hour	48.4	22.5	27.9	7.1	69.2	12.5	52.0	31.8
2 hours	63.7	30.1	25.4	9.5	78.8	32.0	42.9	37.9
4 hours	47.6	58.0	24.7	7.1	110.6	40.0	53.2	36.7
8 hours	108.5	86.5	29.3	16.0	166.4	72.9	79.4	67.2
16 hours	230.0	129.3	95.9	30.1	151.3	158.1	88.1	217.6
24 hours	249.0	142.1	193.3	44.6	248.9	177.5	51.2	120.3
48 hours	153.3	73.8	36.6	18.7	81.4	103.2	62.4	63.9
72 hours	274.3	157.5	113.4	35.0	109.4	7.5	109.9	161.2
96 hours	93.8	74.0	56.5	13.5	44.5	36.7	23.5	34.8

thelium of stomach is not as active as intestine in turnover of protein. Protein samples prepared from duodenum and pancreas together generally had conspicuously higher activities than samples from the lower small intestine. High activity in the pancreas probably is the major reason; for when duodenum was separated from pancreas and protein from the two organs prepared separately, specific activity was two or three times greater for pancreas than for duodenum. For example, at one hour after injection specific activity was 21 counts/min/mg for duodenum and 54 for pancreas in a series of measurements on a group of animals not listed in table 2. In the same series, counts at 8 hours were 81 for duodenum, 159 for pancreas. At 24 hours, counts were up to 102 and 202 respectively. Incorporation into ovary remained relatively low between one and 8 hours but had jumped appreciably at 16 hours. Activity in proteins of liver was usually slightly greater than in samples from kidney up to 8 hours. Beginning at 16 hours, activity in kidney usually surpassed that in liver.

Using the same system as that employed previously (Kemp, '56) the relative order of specific radioactivity was estimated for the protein of the 8 organs considered. Each organ in a given animal was given a rating which indicated its order of activity compared with that of other organs. For example, ventral skin in the animal sacrificed at 5 minutes (table 2) received a top rating of one; stomach with the least activity in this animal rated 8. Ratings for the various organs were first added and then divided by 15 (the number of animals listed in table 2). According to this scheme, the average radioactivity of the proteins from various organs over the 4-day period was: ventral skin (highest), duodenum and pancreas, liver, dorsal skin, kidney, small intestine, ovary and stomach (least).

The order in particular animals or in groups of animals within certain limits of time might vary from this average order. For example, if one considers just the animals killed within the first hour, liver has the highest average rating. In the group of animals killed from two hours to 8 hours, duodenum and pancreas rate highest. Ventral skin had the highest average rating in all animals sacrificed after 16 hours. This changing pattern of radioactivity is probably indicative of the continuous competition for available precursors as metabolic turnover progresses.

DISCUSSION

It was earlier shown (Kemp, '56) that "skin is the most active, and skeletal muscle the least active, with respect to uptake of glycine into proteins of the frog." Pro-

teins of ventral skin proved to be most radioactive in the present investigation, those of stomach least active. Probably the relatively large proportion of connective tissue and smooth muscle in the stomach accounts for its low total avidity for glycine, since the lining epithelium of stomach selectively incorporates glycine. We judge that the localized incorporation of glycine into epithelia of skin, stomach and intestine, into parenchymal cells of liver, and into exocrine cells of pancreas in the frog reflects regions of active synthesis of proteins and possibly also of nucleic acids. Evidence supporting this interpretation is revealed below.

Ficq and Brachet ('56), utilizing phenylalanine-2-C^{14}, showed localizations indicating high protein synthesis in exocrine cells of pancreas, parenchyma cells of liver, and crypts of intestinal glands in the mouse. They also reported localization of high synthetic activity in red pulp of spleen, tubules of kidney and mucosa of uterus. Lung and heart were poorly labeled in their experiments. Karpishka and Carneiro ('60), working with mice, have found that methionine, labeled either with C^{14}, S^{35}, or H^3, is incorporated more readily into sites of cell renewal such as tongue epithelium, or of glandular secretion such as pancreas and submaxillary gland, than into sites of low turnover such as skeletal muscle. Belanger ('56) concludes that methionine or cystine labeled with S^{35} is most readily incorporated into proteins in glandular tissue and areas of intense cell growth in the rat. Leblond, Everett and Simmons ('57) distinguish three types of regions exhibiting high protein synthesis in mammals: (1) those where new cells are forming, (2) secretory glands, (3) sites with high intracellular protein renewal, e.g., kidney cortex and nerve cells. The sites which exhibit high radioactivity in our experiments fall into one or another of these categories.

The question arises how accurately uptake of radioactive glycine, as demonstrated in our experiments, indicates protein synthesis. Can autoradiograms from tissues of animals injected with labeled glycine be used to detect localization of active protein synthesis, as suggested earlier (Kemp, '56)? Valid objections have been raised (Leblond, Everett and Simmons, '57) to the use of glycine for detecting protein metabolism directly, because this amino acid is not utilized exclusively by proteins.

There seems little doubt that radioactive glycine does localize in sites of active protein synthesis even though some of an injected quantity of this amino acid may be used directly for synthesis of nucleic acids (Ficq, '55; Clark, Naismith and Munro, '57; Hayashi and Herrmann, '59) or other compounds. We have observed that skin, duodenum and pancreas, and liver from frogs sacrificed between one and 4 hours after injection gave denser autoradiograms with x-ray film (table 1) than did other organs. Radioactive proteins which had become labeled in these organs (table 2) up to that time might account chiefly for the density of these autoradiograms. Since we did not measure the radioactivity of nucleic acids or other fractions besides protein in our experiments, we cannot tell how much non-protein compounds in our tissue sections contributed to the density of autoradiograms. It is pertinent to mention here that synthesis of nucleic acid and protein appear to proceed simultaneously both in the nucleus (Ficq and Errera, '58) and in the cytoplasm (Clark, Naismith and Munro, *op. cit.*).

Additional evidence that uptake of glycine reveals regions of active protein synthesis is the similarity we have been able to demonstrate between uptake of glycine and methionine. To compare these two amino acids, we injected one group of 14 frogs with 5 μc of S^{35}-methionine and sacrificed them at intervals of 4, 8, 16, 24, 48 and 72 hours. Autoradiograms of tissues from these animals made either on stripping film or x-ray film, showed localizations of radioactivity similar to those in glycine-injected animals. Proteins were isolated from an additional specimen which had been injected with 6 μc of S^{35}-methionine and sacrificed 5 days later. Specific activities (counts/min/mg) and order of activity (number in parentheses after specific activity) of these proteins were: ventral skin, 137.9(1); dorsal skin, 96.0(3); ovary, 4.9(8); stomach, 23.5(7); duodenum and pancreas, 93.5(4); lower small intestine, 67.3(5); liver, 31.4(6); kidney, 105.8(2). Kidney had next to the

highest and ovary the lowest radioactivity in this specimen; otherwise the order of activity of proteins from the various organs was closely similar to that in the 96-hour specimen which had been injected with C^{14}-glycine (table 2). Since order of activity of both ovary and kidney varied even among different animals injected with glycine (table 2), the discrepancies between their relative radioactivities in one animal injected with glycine and another injected with methionine could be ascribed to individual differences in the protein metabolism of ovary and kidney in these two animals.

Incorporation of radioactive amino acids into liver proteins *in vitro* appears to proceed in two steps (Hultin, '56; Hultin and Beskow, '56); (1) accumulation of activated intermediates in the presence of a non-particulate energy-rich fraction of cytoplasm, and (2) introduction of these activated compounds into proteins in the presence of microsomes. Hultin and his collaborators (Hultin, Von Der Decken, and Beskow, '56; Hultin and Bergstrand, '60) have shown that ribonuclease inhibits its uptake of C^{14}-labeled leucine into proteins of homogenates of rat tissues and of sea urchin eggs and embryos. They suggest that protein synthesis may be regulated by the abundance of nucleoprotein particles in cells. Ficq and Brachet ('56) and Martin and Brachet ('59) have called attention to the close parallel between cytoplasmic basophilia and level of incorporation of amino acids. Degree of cytoplasmic basophilia is generally considered to reflect the level of RNA particles, which are predominately bound to rough-surfaced elements of the endoplasmic reticulum (Palade and Siekevitz, '56; Hanzon, Hermodsson and Toschi, '59). We may surmise that abundant RNA particles (ribosomes) partly account for the high level of incorporation of radioactive amino acids reported by Campbell, Greengard and Jones ('57) for liver tumor, ascites tumor and regenerating liver in the mouse. An increase in amino acid-activating enzymes in the cell fluid of regenerating rat liver cells has been hypothesized (Hultin and Von Der Decken, '57). Amino acids after activation may be incorporated into proteins of various fractions of cells including nuclei, mitochondria, microsomes and cell sap (Ziegler and Melchior, '55, '56; Emmelot, '57; Nakano and Monroy, '58; Giudice, '60).

Factors which may influence incorporation of injected amino acids into larger compounds within tissue cells (Henriques, Henriques and Neuberger, '55) include rate of blood flow, rate of penetration of the amino acid into tissue cells, and rate of synthesis of the compounds in question. Junqueira, Hirsch and Rothschild ('55), and Rothschild, Hirsch and Junqueira ('57) could not detect appreciable uptake of glycine into proteins of pancreatic juice of the rat within the first hour after injection. Maximum radioactivity was reached in 2–4 hours. They obtained similar results with labeled glycine, methionine, histidine, alanine and phenylalanine. Using high resolution autoradiography, Mutolo, Giudice and Miceli ('58) could demonstrate uptake of S^{35}-labeled methionine into Ehrlich ascites tumor cells of the mouse within 15 minutes after injection. Ficq and Brachet ('56) report that radioactivity of proteins approached maximum levels within 30 minutes after injection of phenylalanine-2-C^{14} in the mouse. Activity was slightly higher at 75 minutes but tended to decrease after 16 hours except in the kidney, where the level increased.

We have shown by autoradiography with x-ray film that glycine is detectably incorporated into frog skin, liver and kidney of animals sacrificed 5 minutes after injection, and into ovary, duodenum and pancreas, lower small intestine and spleen of animals sacrificed 10 minutes after injection. Incorporation into stomach could be detected by 30 minutes after injection. Although our techniques have not been precise enough to determine the exact time when incorporation of labeled glycine begins in the various organs we have studied, we can say that all of these organs start incorporating the amino acid within 5 minutes to ½ hour after injection. Maximum activity appears to be reached within 24 hours after injection. The fairly close correlation between level of intensity of autoradiograms and the specific activity of corresponding samples of proteins (tables 1 and 2) enables one to conclude that uptake of glycine does roughly reflect protein synthesis. Ra-

dioactivity detected autoradiographically when activity of proteins is very low, however, as in the stomach from 30 minutes to 4 hours after injection (table 2), may be an indication of incorporation of glycine into RNA or other non-protein compounds fixed in tissue sections.

SUMMARY

1. Fifty-three adult female summer frogs were injected intrapleuroperitoneally with 5 μc or 10 μc of C^{14}-glycine and sacrificed at intervals ranging from 5 minutes to 96 hours after injection. Autoradiograms were made by exposing x-ray film, stripping film, or coated emulsion to sections of skin, ovary, stomach, duodenum and pancreas, lower small intestine, liver, spleen and kidney. Proteins were isolated from all these organs, except spleen, and their radioactivity measured by Geiger counting of plated samples.

2. Radioactivity was detected by autoradiography with x-ray film in ventral skin, liver and kidney of an animal fixed 5 minutes after injection. Activity was detectable in all organs except stomach by 10 minutes. The latter acquired detectable radioactivity by 30 minutes after injection. Levels of activity increased at different rates in different organs but had become strong or very strong in all organs by 24–48 hours after injection. Activity remained relatively high up to 72 hours but had declined somewhat in an animal fixed at 96 hours.

3. Distinct localizations of radioactivity were revealed by autoradiograms on stripping film. Epidermis of skin, lining epithelia of stomach and intestine, parenchyma cells of liver, and exocrine cells of pancreas were regions where radioactivity was higher than in other tissues. There was no conspicuous localization in the spleen or in the young oocytes of the specimens studied.

4. Proteins became labeled at different rates in different organs. Their specific activities (counts/min/mg) increased to maximal levels at 24–72 hours and declined by 96 hours after glycine was injected.

5. Incorporation of glycine is correlated with protein synthesis, even though part of the radioactivity detected in autoradiograms may be due to uptake of glycine into nucleic acids or other non-protein compounds.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of sections of tissues stained with methyl green and pyronin, superimposed by autoradiograms on permeable base stripping film. Sections are from organs of one adult female frog injected with 5 μc of C^{14}-glycine and sacrificed 24 hours later. Magnification $\times$ 445.

1 Ventral skin showing high concentration of silver grains (black dots) over epidermis (*e*). Chromatophore (*c*) is in dermis just beneath epidermis. Skin gland (*g*) projects downward into stratum spongiosum of the dermis, where silver grains are much less numerous than in epidermis.

2 Dorsal skin showing similar localization of silver grains over epidermis (*e*). Melanin (*m*) is prominent in a dermal melanophore just below the epidermis. Skin gland (g) projects down into the stratum spongiosum.

3 Basal end of gastric gland (*gg*) showing high concentration of silver grains over zymogenic cells. Muscularis mucosae (*mm*) and submucosa (*s*) are much less radioactive.

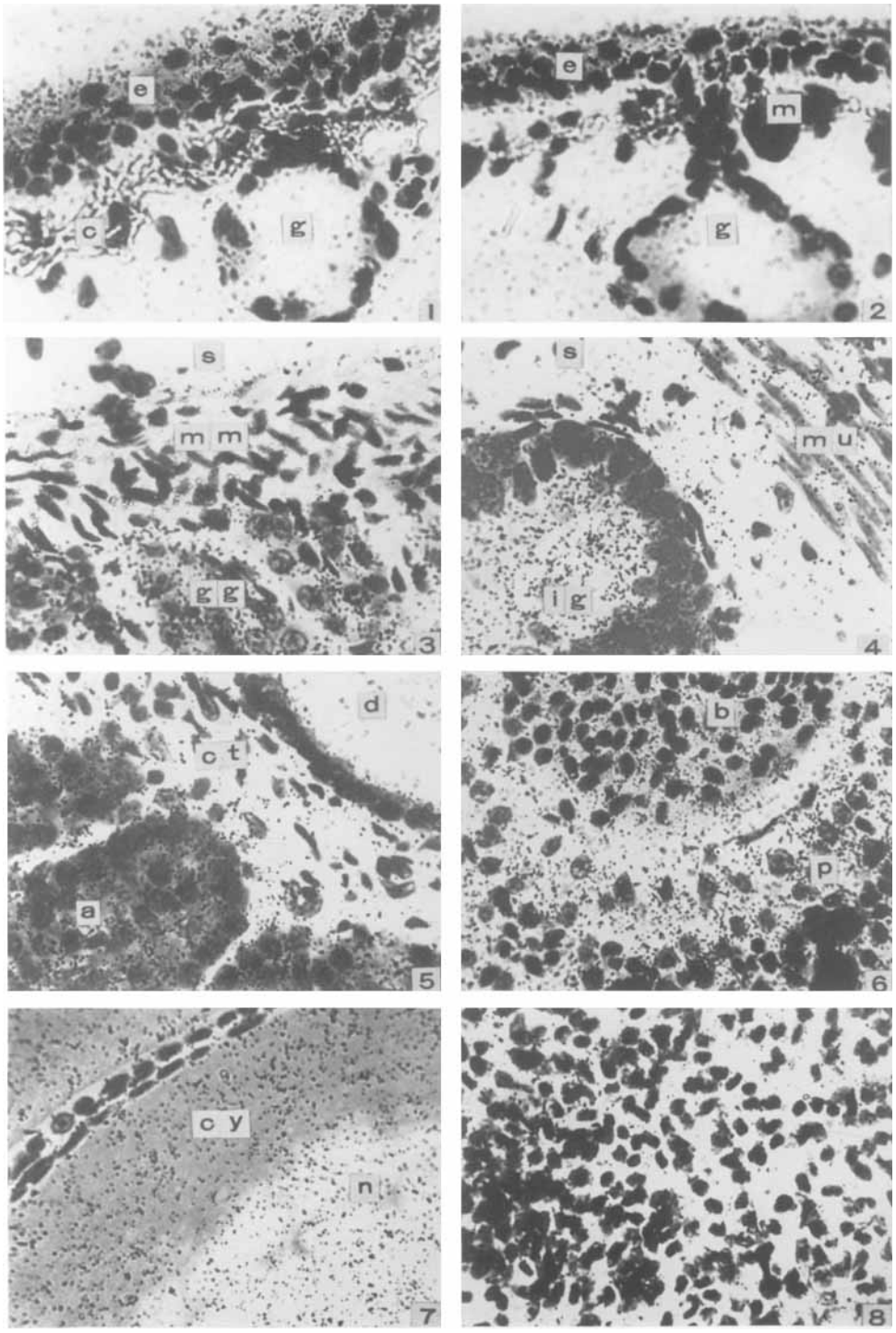
4 View of basal end of an intestinal gland (*ig*) from lower small intestine showing localized high concentration of silver grains over epithelium. Connective tissue of mucosa and submucosa (*s*), as well as smooth muscle of the muscularis (*mu*), shows lower concentration of silver grains and tracks.

5 Exocrine cells of pancreatic acini (*a*) are overlain by high concentration of silver grains. Connective tissue (*ct*) and cells of a duct (*d*) are lightly overlain.

6 Parenchyma cells (*p*) of liver covered by high concentration of silver grains. Massed blood cells (*b*) in blood vessel are moderately radioactive but less so than parenchyma.

7 Periphery of two young oocytes at stage Y^o with layers of follicle cells separating them. There is no conspicuous localization of radioactivity either in cytoplasm (*cy*) or nucleus (*n*).

8 Spleen has relatively low radioactivity throughout. There is no clear localization, although there may be slightly greater activity in the region to the left where the population of lymphoid cells is densest.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