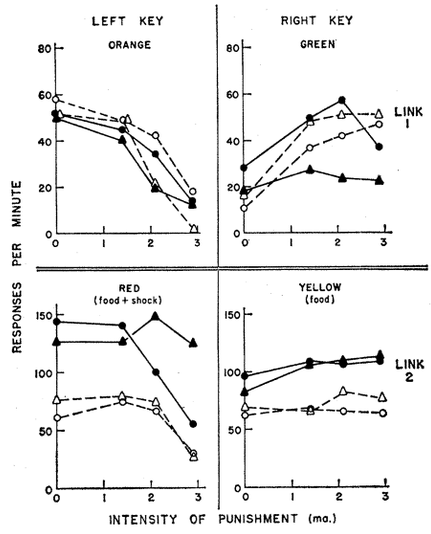

Fig. 1. Rate of pecking on the left and on the right keys during link 1 (upper graphs) and during link 2 (lower graphs) as functions of the intensity of punishment (milliamperes of electric current) following each peck on the left key during link 2. Filled and unfilled points and solid and dashed lines distinguish the effects of different frequencies of positive reinforcement; triangles and circles distinguish the two pigeons.

ments per hour. Each point is the median of three or five successive sessions.

The effect of increasing intensities of punishment on the rate of pecking the left key during link 2 was variable (lower left graph). One curve (filled triangles) shows almost no effect of punishment on the rate of the pecking directly punished. Despite this, the rate of pecking the left key during link 1 (upper left graph), when both keys were lighted, declined regularly as the intensity of punishment increased. This shows the sensitivity of the present method in measuring the effects of punishment, although the change from 72 to 43 reinforcements per hour during link 2 did not produce an expected (*5*) decrease in the rate of pecking during link 1 (filled vs. unfilled points).

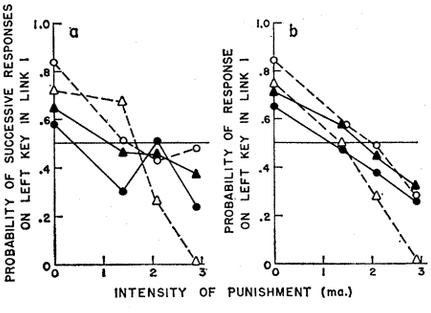

Fig. 2. (*a*) The proportion of pecks on the left key during link 1 for which the next peck was also on the left key; (*b*) the number of pecks on the left key during link 1 divided by the total number of pecks on each of the two keys during link 1, both as functions of the intensity of punishment following each response on the left key during link 2. The symbols are the same as in Fig. 1.

The rate of pecking the right key during link 2 (lower right graph) was relatively constant, but when both keys were lighted during link 1 the rate of pecking the right key generally increased (upper right graph). Thus punishing pecks during the second link on the left key had two effects on behavior when both keys were lighted during link 1: less frequent pecking on the left key where pecks changed the color to one associated with punishment and positive reinforcement and more frequent pecking on the right key where pecks changed the color to one associated with a lower frequency of positive reinforcement.

The change in preference with increasing intensity of punishment was accompanied by a decreasing tendency to peck the left key twice in succession (Fig. 2*a*). The ordinate in Fig. 2*a* is the proportion of pecks on the left key for which the next peck was also on the left key. The symbols are the same as in Fig. 1.

Figure 2*b* summarizes the approximately linear change in preference as a function of the intensity of the punishment. The ordinate is the number of pecks on the left key divided by the total number of pecks on the two keys. At about 1.7 ma, where this statistic has a value of 0.5, there is no preference for either key. That is to say, a stimulus associated with 72 (or 43) reinforcements per hour and 1.7 ma of punishment maintained a rate of pecking on the left key that was approximately equal to the rate maintained on the right key by a stimulus associated with 30 (or 18) reinforcements per hour and no punishment. Punishing and decreasing the frequency of positive reinforcement may thus have the same effect on the reinforcing potency of a conditioned reinforcer, in the sense of decreasing the rate of pecking maintained by presentations of the conditioned reinforcer.

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References and Notes

1. See N. H. Azrin and W. C. Holz, *J. Exptl. Analysis Behavior* **4**, 343 (1961), which contains additional references.
2. Supported in the Harvard Psychological Laboratories by grant M–5139 from the National Institutes of Health. Expenses of preparation paid by grant B–316 from the National Science Foundation to the University of Chicago.
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4. N. H. Azrin [*J. Exptl. Analysis Behavior* **2**, 161 (1959)] describes the technique.
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Serum Protein Synthesis by the Fetal Rat

Abstract. *Injection of C^{14}-labeled amino acids intraperitoneally into rat fetuses* in utero *results in greater labeling of fetal serum proteins than when the amino acid is injected into the mother. Maternal partial hepatectomy leads to only minimal changes in serum protein synthesis. Rat fetuses synthesize at least some of their serum proteins during the last 3 days of gestation.*

There is both genetic (*1*) and isotopic (*2*) evidence that serum proteins are produced by the mammalian fetus. The phenotypes of the haptoglobin and transferrin present in human cord serum may differ from those in the maternal serum, thus indicating their formation by the fetus (*1*). Labeled amino acids have been shown to be incorporated by slices of human fetal liver and by premature guinea pigs in vivo into serum albumin and the serum globulins with the exception of γ-globulin (*2*).

We have previously shown by immunoelectrophoresis that the rat fetus gradually acquires most of the serum proteins present in the adult during the last part of gestation (*3*). This report presents evidence of the production of serum albumin and at least some of the serum globulins by the rat fetus during this period. Effect of maternal subtotal hepatectomy on forming maternal and fetal serum proteins was investigated.

Sprague-Dawley rats in the 20th to 22nd day of gestation were used in all experiments. In one series of experiments, the pregnant rats were anesthetized and the left and median lobes of the liver were removed. The labeled amino acid was injected via the tail vein and the mother and fetuses were bled 6 hours later. Intact and sham-operated rats were injected similarly to

Table 1. Effect of hepatectomy on the incorporation of L-methionine-methyl-C^{14} into maternal and fetal serum globulins. Dose: 10^6 count/min, 37 μg per mother.

Av. fetal wt. (g)	Percentage hepatectomy	Specific activity (count/min per milligram of globulins)	
		Mother	Fetus
2.6	69	34	0
3.1	68	28	41
3.1	Sham control	46	64
	Intact control	41	56

serve as controls. In a second group of experiments the amino acid was injected directly into the fetus *in utero*. The fetuses were located and injected intraperitoneally through the uterine wall with the amino acid. Three hours later the fetal and maternal bloods were obtained. The fetuses showed spontaneous movement when bled. The serums were separated immediately and either processed or frozen at −20°C. The serum proteins were precipitated and washed by the procedure of Zamecnik *et al.* (*4*). Serum albumins of several species are soluble in a number of organic solvents after precipitation with trichloroacetic or other acids (*5*). This property was used to separate the serum albumin from the serum globulins during the washing. The supernatant fluid from the first ethanol wash was saved and either it was exhaustively dialyzed against distilled water or the ethanol was evaporated *in vacuo*. The dialyzed material was electrophoretically homogeneous and showed the mobility of albumin but had at least two quantitatively minor contaminants evident upon immunoelectrophoresis. The albumin samples were prepared for counting by reprecipitation in trichloroacetic acid and heating to 95°C for 15 minutes, which rendered the albumin insoluble in ethanol. The washed precipitates were dissolved in 0.1*N* NaOH and counted in a liquid scintillation counter with the solvent system described by Bray (*6*). Protein concentrations were measured by the procedure of Lowry *et al.* (*7*).

Table 1 presents the specific activities of the fetal and maternal globulins when L-methionine-methyl-C^{14} was injected by tail vein into intact, sham-operated, and partially hepatectomized pregnant rats. The specific activity of the fetal serum globulin was variable and the effect of hepatectomy small. Similar results were obtained when the incorporation of L-leucine into serum albumin and globulins was studied by this method.

Table 2 shows the incorporation of amino acids into the serum proteins of the mother and fetus when the fetuses were injected *in utero*. The consistent finding of a much higher specific activity in the fetal serum proteins than in the corresponding maternal proteins indicates that serum albumin and at least some of the serum globulins are formed by the fetus at this stage of gestation. The marked difference between the two methods of injection may be attributed to the much larger pool of amino acid with which the labeled amino acid is diluted when it is injected directly into the maternal circulation. Further, the transplacental concentration gradient of free amino acids indicates a limited passage of amino acids from the fetus to the mother (*8*). Therefore, an amino acid injected into the fetus would be preferentially utilized by the fetus. Our findings regarding the effect of the route of injection on the utilization of amino acids by mother and fetus are in agreement with those of Kulangara and Schjeide (*9*) in rabbits, although different methods of expressing the results were used in the two studies (*10*).

Table 2. Incorporation of labeled amino acids into serum proteins by the rat fetus. Abbreviations: *A*, albumin; *G*, globulins.

Av. fetal wt. (g)	Fetuses (No.)	Fraction	Specific activity (count/min per milligram of protein)	
			Mother	Fetus
L-methionine-methyl-C^{14} (dose: 10^5 count/min, 3.7 μg per fetus)				
5.3	9	G	13	160
L-leucine-U-C^{14} (dose: 8.06 × 10^4 count/min, 0.52 μg per fetus)				
2.6	18	A	55	484
		G	47	439
3.7	13	A	33	274
		G	18	287
4.2	11	A	53	193
		G	24	183
L-leucine-U-C^{14} (dose: 2.02 × 10^5 count/min, 0.25 μg per fetus)				
3.3	5	A	34	846
		G	29	912
3.8	9	A	68	771
		G	56	803
3.9	13	A	113	822
		G	110	844
4.2	11	A	77	474
		G	52	446
4.6	11	A	78	417
		G	49	418

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10. Aided by grants from the Charles and Marjorie King Fund and from the Association for the Aid of Crippled Children, and by training grant 2G-144 from the National Institutes of Health.

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Intracellular Potentials of Cortical Neurons during Focal Epileptogenic Discharges

Abstract. *Focal electroencephalographic discharges in lesions of cortex induced by freezing are associated with prolonged membrane depolarizations and hyperpolarizations in neurons located at various depths in the lesion sites. Transmembrane potential changes have properties similar to those of postsynaptic potentials. The temporal relationship between intracellular potentials and paroxysmal discharges indicates that the latter are extracellularly recorded summations of synchronously developing depolarizations and hyperpolarizations in complex synaptic organizations of neurons.*

The focal electroencephalographic spike and sharp wave represent elementary electrographic abnormalities observed in clinical electroencephalography (EEG). Although recent reviews have stressed the possible relationship of paroxysmal activities to extracellularly recorded unit discharges (*1*), we do not know to what extent the different electrical responses of neurons contribute to the production of cortical surface EEG spikes. Intracellular recording from cortical neurons has revealed membrane depolarizations and hyperpolarizations during different stages of strychnine-induced seizure discharges (*2*). These responses have been attributed to strychnine potentiation of spontaneous oscillations of membrane potential. Some temporal correlations have been noted between