has seen no spontaneous ovulation between June and September, although he has performed at least 50 laparotomies during these months. This fact agrees with the published findings of Hartman(9). While the results reported here are presented with the full realization that they are not conclusive, the author feels that they do compare very favorably with the better results of experimentation on ovulation in the monkey by other means. The single ovulation in the summer of 1948 might possibly be considered a chance occurrence. However, it is felt that the number of ovulations in 1949 were too frequent to be due to chance.

Accepting the above results to mean that progesterone produces ovulation in the monkey during the anovulatory summer period, provided a follicle of sufficient size is present, the monkey can be added to such other species as the rat(6) and the chicken(7), in which progesterone causes ovulation. It is also probable that the ovulation is the result of the release of LH from the hypophysis intermediated by the progesterone(10,11). This interpretation fits very well with the finding that progesterone is produced by the preovulatory follicle in all mammals in which it has been tested. The presence of progesterone has also been detected in the blood of the monkey(12) and of women(13) before ovulation.

It is interesting that the next bleeding seemed to occur in relation to the last injection of progesterone and not to the presence or absence of a corpus luteum. The apparent lack of correlation between the length of progesterone treatment and the number of days before menstruation(14) is probably not significant since the material was not adequately controlled for this factor.

Summary. Ovulation occurred in 4 of 11 adult female monkeys which had received 0.5 mg of progesterone daily for 3 to 6 days beginning on the 10th to the 14th day of the menstrual cycle during the anovulatory summer period. Menstruation began from 3 to 8 days after the cessation of the progesterone treatment whether ovulation occurred or not.

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Free Glutamic Acid Content of Viridans Streptococci and Some Trained Resistant Strains.* (18231)

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Gale(1) and Taylor(2) have determined the internal glutamic acid content of many different species of bacteria. Gale(3) has shown that sulfathiazole interferes with the condensation of glutamic acid into bacterial protein. Since the assimilation from the medium into the cell was not blocked, it might be expected that glutamic acid would accumulate in the cell in the presence of sulfathiazole. Gale found this to be true. He also reported(4) that resistance to sulfa-

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thiazole in *Staphylococcus aureus* appeared to be accompanied by a large amount of free glutamic acid. As evidence to support this statement he compared the values found for two strains of susceptible and two strains of naturally-resistant *S. aureus*. In previous investigations of the properties of viridans streptococci, we have trained strains of this group to resistance to sulfathiazole(5). Later, strains were also trained to increased resistance to penicillin, aureomycin,‡ and streptomycin. The penicillin and aureomycin-resistant strains were highly resistant to sulfathiazole, but the streptomycin-resistant strains were usually only slightly changed in their susceptibility to sulfathiazole(6). This paper reports the findings of an investigation of the free glutamic acid content of these resistant variants and the parent strains. The results would appear to have added interest in view of the fact that it has recently been found in our laboratory that trained sulfathiazole-resistant variants require pteroylglutamic acid, while the parent strains do not(7).

Experimental. Nine strains of viridans streptococci, 7 strains trained to sulfathiazole resistance, 6 to aureomycin, 5 to penicillin, and 6 to streptomycin resistance, and 2 enterococci were used for the determinations of glutamic acid content. One enterococcus was obtained from the American Type Culture Collection. The other enterococcus and the 9 viridans streptococci were obtained from nose and throat cultures, urine, and pus. They were classified by biochemical tests. The resistant strains were derived from the viridans group by culturing in increasingly higher concentrations of the inhibiting agents in broth. The inhibiting concentrations for all organisms were determined by inoculating 5 fold dilutions of the agents in tryptose phosphate broth, with 1 drop of a 1-1,000 dilution of a 24-hour broth culture. Differences in turbidity of broth cultures of parent and resistant strains were equalized before diluting. All tests were repeated at least 3 times. The inhibiting concentration was the lowest concentration completely preventing growth. The free glutamic acid content of the cells was determined by the method developed by Gale(8). This method depends upon the manometric measurement of CO_2 liberated from the amino acid by the specific decarboxylase for the acid. A culture of *Clostridium welchii,* SR 12, served as the source of the decarboxylase. Each organism, the glutamic acid content of which was to be determined, was grown in 300 ml of tryptose phosphate broth with .1% yeast extract, and harvested at various times, washed twice with distilled water, and finally suspended in about 4 ml of distilled water. This suspension was divided into 2 parts. One part was used to determine any free glutamic acid carried down with the cells from the medium. The other was placed in boiling water for 10′ to release the glutamic acid inside the cell. One ml of cell suspension and 1.2 ml of M/5 acetate buffer pH 4.5 were measured into the main compartment of a Warburg flask and 1 ml of the decarboxylase preparation was placed in the side arm. The CO_2 evolved from the boiled cells less that from the control was expressed in microliters of glutamic acid, as Gale(8) has done for his values. (The values may be changed to micromoles of glutamic acid by dividing by 22.4.) The weight of the cells used was determined by drying an aliquot of the suspension and weighing.

Results and discussion. Table I shows the increase in resistance attained by growing the parent strains in increasing concentrations of sulfathiazole, aureomycin, penicillin, and streptomycin. The increase in resistance to sulfathiazole found in the aureomycin, penicillin, and streptomycin-resistant strains is also given in order to point out the correlation between the internal glutamic acid content and the resistance to sulfathiazole. All

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TABLE I. Increase of Resistance in Trained Strains to Sulfathiazole, Aureomycin, Penicillin, and Streptomycin and the Concomitant Increase in Resistance to Sulfathiazole.

	Resistant variants of						
Culture No.	Sulfathiazole Fold increase to Sulfa	Aureomycin Fold increase to Aureo	Aureomycin Fold increase to Sulfa	Penicillin Fold increase to Pen.	Penicillin Fold increase to Sulfa	Streptomycin Fold increase to Strepto.	Streptomycin Fold increase to Sulfa
1	300	100	300	400	200	200	5
4	300	100	300	1000	300	200	5
6	300	300	300	400	300	200	5
17	300	2000	300	1000	300	5000	5
23	300					1000	5
27	30	100	30				
57	30	100	300	1000	300	10,000	30

TABLE II. Free Glutamic Acid Content of Parent and Resistant Cells.

	Culture No.	Hr of growth at 37°C					
		6	8	10-12	16-18	24	36
		μl/100 mg cells					
Viridans	1		0	0	0	0	
	4	0	0	0	0	0	
	6		0	0	0	0	
	17			0	0	0	
	23					0	
	27					0	
	57					0	
	18		0	0		0	
	46		0	0	0	0	
S. fecalis	9790	163	246	205	287	263	
	47	156	260	162			
Sulfathiazole—r.v.*	1	168	220	145	120	105	
	4	142	128	181	92		
	6	226	311	203	118	68	
	17	134		168	114	284	
	23		118	180	113		
	27		170	201	135		
	57	284	399	353	162		
Aureomycin—r.v.*	1		347	351	270		
	4		208	234	80		
	6		258	262	235		
	17	158		187	174		
	57	179	333	234	139		
Penicillin—r.v.*	1		129	175	107		
	4		372	378	360		
	6		301	234	161		
	17	290		204	140		
	57		308	328	183		
Streptomycin—r.v.*	1				0	0	0
	4				0	0	0
	6				0	0	0
	17				0	0	0
	23				0	0	0
	57		388	309	147		

* Resistant variant.

of the strains grown in sulfathiazole, aureomycin, and penicillin have increased 30-fold or more in their resistance to sulfathiazole. Only one of the 6 strains trained to resistance to streptomycin showed a 30-fold increase to sulfathiazole. This was the strain which increased 10,000-fold in resistance to streptomycin. The other strains showed only a slight increase in sulfathiazole resistance.

Table II records the microliters of glutamic acid per 100 mg of dry cells found in the parent and resistant strains, and in 2 strains

of *Streptococcus fecalis*. Preliminary determinations had shown that the greatest amount of glutamic acid was found just at the time the cells had reached the end of active growth. This point was determined by following the increase in turbidity of the broth culture with the Klett-Summerson photoelectric colorimeter. In order that the time would not be missed when the greatest amount of glutamic acid would be present, several determinations were made at various times before and after the end of active growth. Each value given is for a single determination. Repeated determinations made on several of the organisms gave some variation in values at a given time of harvesting, but those showing positive values were always positive, and those showing negative values were never found to be positive. It is interesting that, under the conditions of this experiment, no free glutamic acid was found in any of the gram positive viridans streptococci. Taylor (2) reported very low values for some, but no completely negative values for any gram positive organism. His manometric determinations were made at 30°C, which Gale(7) reports as optimum for decarboxylase activity. Our determinations were made at 37°C. The values found for our known strain of *S. fecalis* were comparable to those found by Gale(1) for *S. fecalis*.

Both strains of *S. fecalis* and all of the 8 strains trained to sulfathiazole resistance by growing in sulfathiazole showed a high internal concentration of free glutamic acid. All of the trained aureomycin and penicillin-resistant strains also showed a high internal concentration of glutamic acid, but only the one streptomycin-resistant strain which had a concomitant 30-fold increase to sulfathiazole showed the presence of free glutamic acid. The other streptomycin-resistant variants, whose sulfathiazole resistance increased only 5-fold, gave negative results when determinations for glutamic acid were made. High values for internal glutamic acid apparently accompany resistance to sulfathiazole, whether this is natural resistance, resistance induced by growing in sulfathiazole, or resistance accompanying an increase of resistance to other agents.

Other studies in our laboratory, to be reported later(6) have shown that the same biochemical properties are seen in trained sulfathiazole, aureomycin, and penicillin-resistant strains, but these properties are often not found in trained streptomycin-resistant strains. These are properties characteristic of enterococci. In our studies, glutamic acid in concentrations similar to those found in *S. fecalis*, were found in the resistant cells.

Gale(4) has made the suggestion that the large amount of glutamic acid in resistant cells may itself be responsible for the increased resistance to sulfathiazole, since he found that in media containing graded amounts of glutamic acid, the media with the most glutamic acid required the most sulfathiazole to prevent growth of *S. aureus*. It may be, however, that the resistant cells become so because of a change in metabolism which eliminates the necessity of using the free glutamic acid taken up by the cell from the external environment, and so allows it to accumulate in the cell. Since it has previously been shown(7) that trained sulfathiazole-resistant strains of *Streptococcus mitis* require pteroylglutamic acid (PGA), while the parent susceptible strains do not, one possible explanation for part of the increase in glutamic acid might be that the resistant cells no longer are able to use free glutamic acid for the synthesis of PGA. The amount needed for this synthesis would hardly be enough to account for the differences noted here between susceptible and resistant cells, however. Some other products needed by the cell, and synthesized from free glutamic acid in the parent susceptible strains, may be supplied from another source in the resistant cell and allow more accumulation of the glutamic acid. Further information on this subject might be obtained by employing Gale's technics for preventing internal metabolism, and measuring the effect on the glutamic acid content of the susceptible cells, since at present we do not know whether these cells use glutamic acid from the environment or synthesize it themselves.

Summary. Free glutamic acid was found in all of the trained sulfathiazole, aureomycin,

and penicillin-resistant strains of viridans streptococci tested, but not in the parent strains. The aureomycin and penicillin-resistant strains had also increased in sulfathiazole resistance. No glutamic acid was present in 5 trained streptomycin-resistant strains which had not increased appreciably in sulfathiazole resistance, but was found in one strain which had increased in sulfathiazole resistance. Glutamic acid appears to accompany sulfathiazole resistance regardless of how this resistance is acquired. The possible relation of the free glutamic acid to the increased resistance to sulfathiazole is discussed.

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Levels and Intracellular Distribution of Coenzyme A and Pantothenic Acid in Rat Liver and Tumors.* (18232)

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The relative inability of tumor homogenates to oxidize oxalacetic acid(1,2) and the participation of coenzyme A (CoA) in the enzymatic condensation of oxalacetate and C_2 units to citrate(3,4) suggest that tumors might be relatively deficient in this coenzyme. Earlier work pointed to a low level of pantothenic acid (PA) in tumors(5), but the methods available at that time for liberating the vitamin from bound forms were probably inadequate(6). Therefore the contents of coenzyme A and pantothenic acid were determined in several types of transplantable rat tumors. Since the enzymes involved in the Krebs tricarboxylic acid cycle appear to be relatively concentrated in the mitochondria of normal liver(7) a comparison was also made of the intracellular distribution of these factors in normal rat liver with that in azo dye-induced rat liver tumors.

Experimental. The transplantable tumors were collected from multiply-inoculated rats† bearing the Walker carcinosarcoma, the Jensen sarcoma, or the Flexner-Jobling carcinoma. Only firm tumors less than 1 cm in diameter were analyzed. The minced tumors were homogenized in ice-cold isotonic saline in the Potter-Elvehjem apparatus to give a final tissue concentration of 10%. The liver tumors were taken from rats fed for 5 months on a low riboflavin, semi-synthetic diet(8, diet 3) containing 0.06% 4-dimethylaminoazobenzene. The liver tumors were perfused *in situ* with ice-cold isotonic saline by injection into the aorta at the thoracic level; the aorta below the coeliac artery was clamped off and the fluid allowed to escape by severing the vena cava above the liver. This perfusion route was necessary since liver tumors, unlike liver, are supplied only by arterial blood

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