fering from a cold or similar indisposition. Neither were tests made during menstruation. By coincidence none of the subjects were smokers.

Samples of blood were taken after 15 hours' fasting and collected from a fingerprick into a paraffin well. Folin's micro method[3] was used for the glucose determinations, using 1.6% sodium carbonate in place of the sodium cyanide-sodium carbonate solution as recommended by Klendshoj and Hubbard.[4] Readings were made on a Beckman spectrophotometer at 5200 Å. All measurements and readings throughout the study were done by the same analyst. Periodic standard sugar curves were made to check the solutions and the instrument. A search of the literature revealed only 2 observations[5,6] which suggest a relationship of season to concentration of blood sugar in human subjects.

The results obtained in the study reported here present the question whether these seasonal differences are due to variation in the quality or quantity of the food ingested or difference in the individual metabolism, or both, at different seasons of the year.

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Hearing in Guinea Pigs Deficient in the Anti-Stiffness Factor.* (17367)

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This research was outlined to establish whether a diet deficient in the anti-stiffness factor[1-5] produced diminution in the hearing ability of guinea pigs. Responses of guinea pigs to sound are various. The most frequently observed reflex is the well-known ear-flick which, when well developed, is a folding back of the ear against the side of the head, but in diminished form is a mere quiver of the edge of the ear. This type of response was chosen as the most clear-cut obtainable to establish the hearing range of guinea pigs, although certain animals gave evidence by jerking or movement of whiskers that they heard a little beyond the limits set by the ear-flick.

Stimuli were furnished by a General Electric audio-signal generator combined with a loud speaker and key (borrowed through the kindness of Prof. E. A. Yunker, Department of Physics, Oregon State College). Free response was obtained by placing the unconfined animal upon the table, closely facing the loud speaker. Each guinea pig was exposed to a series of signals from a frequency of zero to a frequency of 17,000 cycles per second. The range in pitch to which ear-flick response was given was found to be 150-16500 cycles per second. Fluctuations in electric current from time to time produced slight variations in response.

The 68 experimental animals used in this research had been fed a diet deficient in the anti-stiffness factor for at least 11 months before the testing started. This diet consisted of 18-20% skim milk powder in water, to which was added copper and iron salts and the following assortment of vitamins: The

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salts and ascorbic acid, 10 mg per animal, were added to the morning feeding. The fat soluble and the water soluble vitamins were added alternately to the evening feeding, allowing 2 cc of the solutions for each animal.

	Vitamin solutions	
Water soluble	Thiamine hydrochloride	0.2 g
1 liter	Riboflavin	0.5
	Pyridoxine hydrochloride	0.1
	Nicotinic acid	1.0
	Calcium pantothenate	0.1
	Inositol	10.0
	p-Amino benzoic acid	2.0
	Choline hydrochloride	50.0
	Folic acid	0.003
	Biotin concentrate	3 drops
Fat soluble	Beta carotene	20 cc
1 liter	Viosterol	10 cc
	Alpha tocopherol	0.1 g
	2-CH_3-1, 4-naphthaquinone	0.1 g

The 68 deficient animals were compared with 68 non-deficient stock guinea pigs which had been fed a diet of rolled barley, with abundant green feed daily. Both groups of animals were bedded in wheat straw and provided with iodized salt and water.

Both stock and deficient animals were subjected to frequent hearing tests over a period of 7 months. In both groups it was found that individuals differed from one another in hearing to some degree, but this difference involved almost exclusively the lowest extent of the hearing range, up to about 2000 cycles per second. The upper limit of hearing with apparently normal animals was very regularly somewhat above 10000 cycles per second.

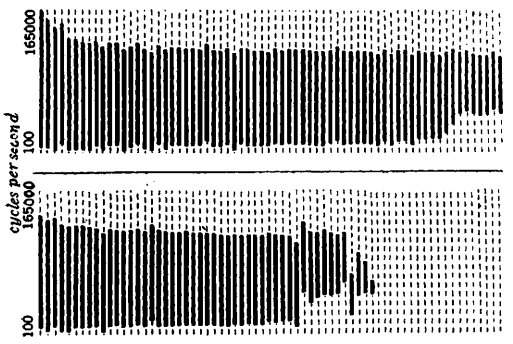

FIG. 1.

Upper series represents hearing range of 68 guinea pigs fed stock diet. *Lower* series represents hearing range of 68 guinea pigs fed deficient diet. Length of individual lines indicates range in pitch of audio-signals from 100 to 16,500 cycles per second. Solid portion of line indicates actual range of each animal. Note (1) that no stock animal had seriously diminished hearing range; (2) 19 deficient animals gave no auditory response.

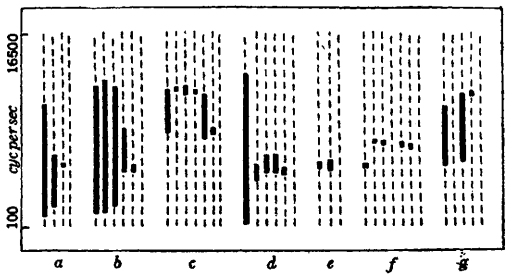

FIG. 2.

Seven individual cases of deficient guinea pigs showing recession of hearing during an experimental period. Length of individual lines indicates range in pitch of audio-signals, 100 to 16,500 cycles per second. Solid portion of line indicates actual hearing range of deficient animal on day tested. Each group of lines represents successive hearing responses of one animal. The decline of each animal to final deafness is shown by an overall decrease in length of solid lines. Final dotted line in each group shows entire absence of hearing response, which was proved by many subsequent tests to be permanent deafness.

Intervals in days between testing dates were as follows: (a) 30, 30, 23, (b) 30, 45, 23, 14, 8, (c) 30, 45, 10, 4, 3, 16, (d) 30, 45, 23, 10, 4, (e) 30, 45, (f) 30, 35, 10, 4, 3, 17, (g) 30, 35, 17, 16.

The 68 non-deficient animals showed normal range of ear-flick response at each test over the 7-month period of observation, the most restricted range being 2,000-10,000 (Fig. 1, upper). Of the 68 deficients tested, 11 never gave ear-flick response at any time during the 7-month period. It should be noted that these animals had been on deficient diet for at least 11 months before the first test. By the end of the 7-month experimental period, 8 more guinea pigs had become deaf (gave no ear-flick response), making a total of 19 deaf animals (Fig. 1, lower). The advancing deafness of 7 of these individuals is illustrated in Fig. 2.

Statistical analysis was made of data on hearing ability of 56 stock animals and 64 deficient animals. The average ranges (highest frequency at which ear-flick was obtained less lowest frequency at which ear-flick was obtained) are given in Table I. The analysis of variance shows that the animals which were fed with the same diet differed from one another in range of response to audio-signals ($F = 34.75$ with 118 and 429 degrees of

TABLE I.
Average Range of Hearing.

	Stock	Deficient
No. of animals	56	64
No. of measurements	176	373
Avg range	10,630	7,328

freedom). It also shows that animals fed stock diet had significantly wider range of response to audio-signals than those fed deficient diet ($F = 18.34$ with 1 and 118 degrees of freedom).

Summary. Guinea pigs fed a diet deficient in the anti-stiffness factor may ultimately develop inability to respond with the ear-flick to auditory stimuli. At the conclusion of the experiment, 28% of the deficient animals were deaf according to the ear-flick test, in contrast to 0% among the stock animals. It was possible to trace developing deafness according to the ear-flick test in a considerable number of animals maintained on a deficient diet during the experimental period of 7 months.

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Evidence that Copper Acetate Induces Ovulation in the Rabbit by Direct Stimulation of the Adenohypophysis. (17368)

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Copper salts induce ovulation in the estrous rabbit,[1,2] a species which normally ovulates only after copulation. The natural mating stimulus, which results in the release of luteinizing hormone from the adenohypophysis, involves the hypothalamus and the pituitary stalk[3] and includes both adrenergic and cholinergic components.[4]

Bischoff[5] suggested that copper activated the hypophysis by toxic stimulatory effects on the nervous system. The idea appeared to be confirmed by the results of Brooks,[3] who found that copper failed to induce ovulation in rabbits whose hypophyseal stalks had been severed, and Harris,[6] who reported that very weak dosages of copper acetate injected directly into the third ventricle stimulated the ovulatory response.

We were led to doubt that copper-induced ovulation is purely a nerve-stimulation phenomenon when dibenamine and atropine, in dosages adequate to block the copulation stimulus from reaching the hypophysis,[4,7] failed to prevent copper acetate from activating the release of an ovulatory surge of LH.[8] It therefore seemed desirable to test the hypothesis that copper might exert its stimulatory action directly upon the anterior pituitary.

Sexually mature female rabbits ranging in weight from 2.5 to 4.3 kg were employed in this study. To insure an estrous condition, each animal was treated with 85 μg estradiol benzoate on 2 successive days prior to copper administration, for ovulation is not induced by copper in anestrous rabbits.[9] A control series of 10 females revealed that the estrogen alone would not stimulate LH release. The hypophysis was approached parapharyngeally as in earlier studies,[10,11] and 4 attempts were made at 5 or 10 minute intervals to inject a total of 0.15 ml of 0.1% copper acetate, buf-

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