

with glutamic acid. The results of these studies are shown in Table II and Fig. 1. From these data it can be seen that pantothenate stimulated respiration considerably when either α -ketoglutarate or glutamic acid was used as a substrate, but succinate, fumarate, oxalacetate and aspartic acid were oxidized to lesser extent. It will be noted that the stimulation values for most of these substrates are in the same range as was observed with pyruvate. It was also possible to demonstrate pyruvate in the vessels after all of these substrates had been oxidized. Thus it can probably be assumed that pantothenate stimulation of certain amino acids and other substrates is due, in part at least, to their being converted to pyruvate which is in turn stimulated. However, the pantothenate stimulation observed when glutamic acid or α -

ketoglutarate was used as substrate cannot be accounted for entirely as being due to pyruvate stimulation alone. Therefore it appears that pantothenic acid also functions in some other way in the oxidation of these substrates. Since the values observed with glutamic acid were very close to those noted with α -ketoglutarate, it would seem to eliminate the possibility of pantothenic acid functioning in the deamination of glutamic acid.

Summary. From this study it can be concluded that the pantothenate stimulation of amino acid oxidation by *Proteus morganii* is limited to those amino acids which can be converted to pyruvate. However, in the case of glutamic acid, and its metabolic intermediate α -ketoglutarate, the pantothenate stimulation appears to be more than can be attributed to pyruvate oxidation.

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Testicular Degeneration as a Result of Microwave Irradiation.

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The mammalian scrotum has been established as being a local thermoregulator for the testes. Moore and Quick¹ found the scrotal temperature of white rats to be from 2° to 8°C lower than the temperature of the abdominal cavity. A sub-abdominal temperature in the scrotum has been shown to be necessary for the continuance of spermatogenesis. Moore² confined the testes of guinea pigs in the abdominal cavity for varying periods of time and found that an abdominal retention of seven days resulted in a complete disorganization of the germinal epithelium of the seminiferous tubules. He considered the cause of this degeneration to be due to the higher temperature of the abdominal cavity.

Investigations of Moore³ showed that testic-

ular degeneration resulted from a single application of heat at approximately 7°C above body temperature for a 15-minute period. The heating devices which were used consisted of hot water baths, electric stoves, electric light bulbs, and hot water pads. Testicular degeneration was visible histologically within four to six days following the heat application, and was entirely similar in type to that resulting from early experimental cryptorchidism.

Fukui⁴ exposed the scrotum of rabbits to sunlight and to warm air, and found a definite relationship between the temperature and the time required to cause regressive changes in the germinal cells. The minimum scrotal temperature at which he was able to produce testicular damage was 40°C. The time of required exposure at this temperature was more than one hundred hours.

It was the purpose of this study to deter-

¹ Moore, C. R., and Quick, W. J., *Am. J. Physiol.*, 1924, **68**, 70.

² Moore, C. R., *Am. J. Anat.*, 1924, **34**, 269.

³ Moore, C. R., *Am. J. Anat.*, 1924, **34**, 337.

⁴ Fukui, N., *Japan M. World*, 1923, **3**, 27.

mine the effect of 12 cm electromagnetic radiations of testicular tissue. In addition, attempts were made to confirm the results of previous investigators relative to the effects of infra red irradiations on testes.

Procedure. Male albino rats of the Sprague-Dawley strain, ranging in age from 120 to 200 days were employed in this study. In preparation for irradiation the animals were anesthetized with ether and the scrotum swabbed with 95% alcohol. Each animal was then arranged on a platform behind a copper shield, and the scrotum was inserted through an opening provided in the shield. Thus, the remainder of the animal was protected from the radiations. An iron-constantan thermocouple needle, of the type described by Tuttle and Janney,⁵ was then inserted into the center of one of the testes. This served to register the degree of temperature produced. The thermocouple had been calibrated previously with a Bureau of Standards thermometer. Thermocouple potentials were measured by a Leeds and Northrup potentiometer. Temperatures were read to the nearest tenth of a degree centigrade. The temperature of the testes was allowed to fall to approximately 29°C before the irradiations were started. Because of possible damage to the testes resulting from thermocouple needle puncture, one testis was employed for temperature measurements and the contralateral testis was used to study the histologic effects of the radiation. Care was taken to align both testes at an equal distance from the source of radiation. In preliminary experiments it was found that if these precautions were taken the temperature rise due to the radiations was the same in both testes.

A Raytheon Microtherm generator (model CMD4) which produced a wave length of approximately 12 cm was used to apply the high frequency radiations. A variac was provided by means of which the power output was regulated. The corner type reflector was used. The infra-red source rated at 600 watts was of the non-luminous type, and a 9-inch hemispherical reflector was employed. In all

cases irradiation was applied to the testis through the scrotum.

Two series of experiments were performed with 12 cm electromagnetic waves. In the first of these series the procedure consisted of elevating the testicular temperature to levels of 47, 46, 45, 44, 43, 40, 37, 35, 34, 33, 32, 31 and 30°C. For each temperature level 4 animals were used, and the testicular temperature was maintained at the selected temperature for a single period of 5 minutes. The temperature was maintained by varying the power output of the microwave machine. From each group of 4, an animal was sacrificed at 4, 8, 12 and 16 days following the exposure and the testes were prepared for histologic examination.

In the second series of irradiations 2 groups of 10 animals were given single 15-minute exposures at temperature levels of 33° and 34°C. A third group of 10 animals was exposed to 35° C for a period of 10 minutes. All animals were sacrificed for histologic studies 4 days following the exposures. The testes were fixed in Bouin's solution and the sections stained in hematoxylin-eosin.

Infra-red irradiation was used in two series of experiments. In the first series testicular temperatures were raised by irradiation to levels of 33, 37, 40, and 43°C for periods of 5 minutes. Four animals were exposed at each temperature level and a histologic examination of the testes was made at 4, 8, 12, and 16 days after the exposures. The procedure in the second series of infra-red irradiations was to expose two groups of 10 animals to temperature levels of 38° and 40°C for 10 minutes. Four days after exposure the animals were sacrificed and the testes were removed for histologic study.

The testes of 12 normal non-irradiated males were employed as histologic controls.

Results. The results of experiments with 12 cm electromagnetic irradiations are listed in Table I. These results show that in all cases when the testicular temperature was raised to 35°C or higher there was evidence of testis tissue damage. At temperatures from 31° to 35°C approximately 50% of the testes showed signs of degenerative changes. The

⁵ Tuttle, W. W., and Janney, C. D., *Arch. Phys. Med.*, 1948, **29**, 416.

TESTICULAR DEGENERATION

TABLE I.
Effect of 12 Cm Irradiation on Male Gonads.

No. animals exposed	Exposure temp. °C	Time of exposure min.	Animals with testicular damage	% with testicular damage
4	47	5	4	100
4	46	5	4	100
4	45	5	4	100
4	44	5	4	100
4	43	5	4	100
4	40	5	4	100
4	37	5	4	100
4	35	5	4	100
4	33	5	2	50
4	32	5	1	25
4	31	5	1	25
4	30	5	0	0
13	35	10	13	100
4	33	10	1	25
4	31	10	1	25
3	35	15	3	100
7	34	15	3	43
16	33	15	9	56
4	31	15	0	0

TABLE II.
Effect of Infra Red Irradiation on Male Gonads.

No. animals exposed	Exposure temp. °C	Time of exposure min.	Animals having testicular damage	% with testicular damage
3	43	5	2	67
4	40	5	2	50
4	37	5	0	0
4	33	5	0	0
8	40	10	3	38
10	38	10	0	0

testes of animals exposed to 30°C were not affected.

The results of experiments with infra red irradiation (Table II) show that testes from 67% of the animals exposed to 43°C were damaged. Testicular degeneration was not found in the experiments where the temperature of the testes was maintained at 38°C for 10 minutes by infra red. No damage was found in the testes of control animals.

A typical histological picture of testicular degeneration following exposure to electromagnetic waves shows an area of degeneration along the side of the testis nearest to the source of radiation (area A, Fig.1). Continuing from this area of degeneration to the opposite side of the testis, all gradations from completely degenerated to normal tubules can be found (areas B and C, Fig. 1).

Fig. 2 clearly shows the transition of degeneration from area A to area B of Fig. 1. These areas are well defined. A detailed picture of a degenerating tubule in area B is shown in Fig. 3. In area A, which was nearest to the source of radiation, complete coagulation of the tubules was found (area A of Fig. 2). This area showed an absence of germ cell nuclear material, the cytoplasm appearing much more refractile than normal cytoplasm. The tissue resembled that seen in burn necrosis. In general the tubules in area B show sloughing of degenerating germinal elements into the lumen, multinuclear masses termed "giant cells" which apparently consist of fused spermatid nuclei, and usually absence of spermatozoa (area B of Fig. 2 and 3). Sertoli and interstitial cells apparently remain intact. Leucocytic infiltration of the

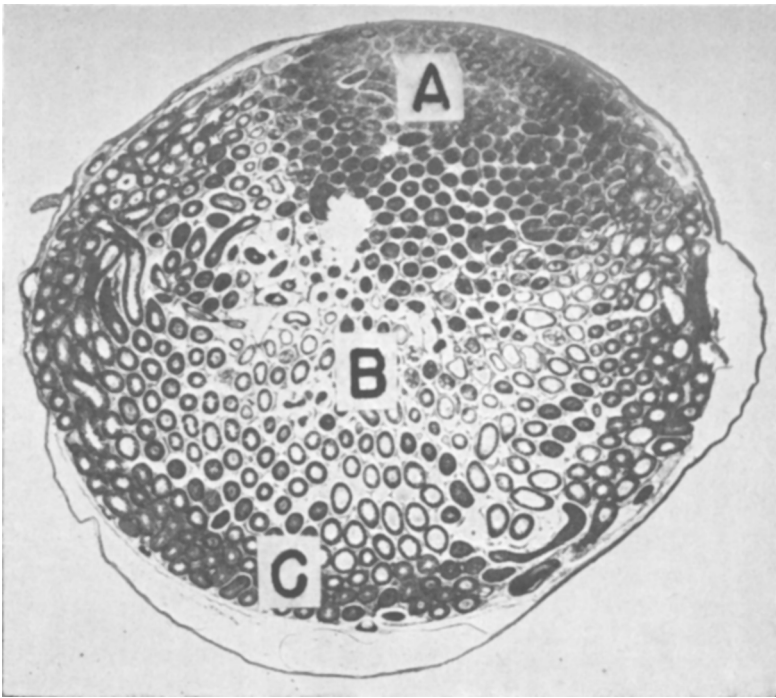


FIG. 1.

Cross-section of entire testis removed 4 days after a single irradiation with 12 cm microwaves at 35°C for 10 minutes ($\times 10$). See text for explanation of the areas indicated.

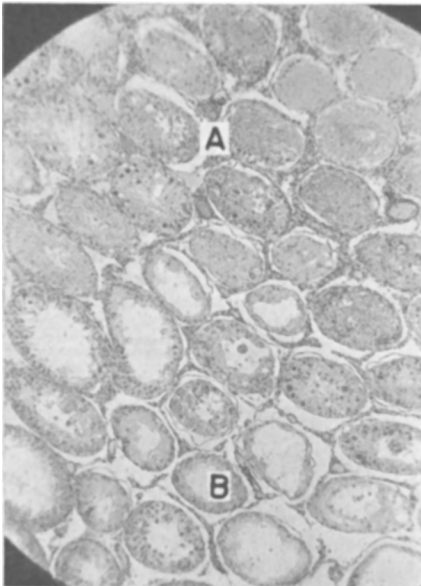


FIG. 2.

Seminiferous tubules from areas A and B of Fig. 1. ($\times 40$).

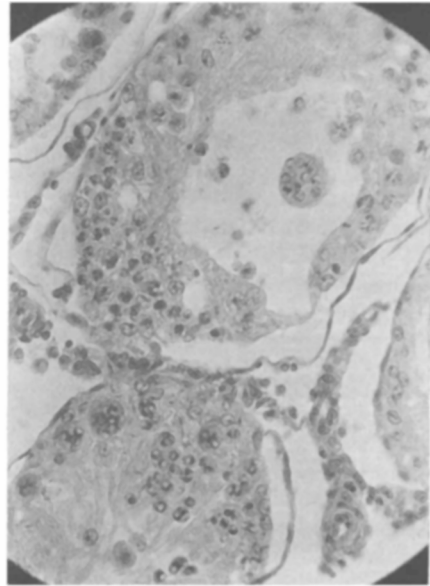


FIG. 3.

High magnification of a degenerating seminiferous tubule from area B of Fig. 2. ($\times 200$).

tissue was evidenced by the presence in some cases of intertubular polymorphonuclear leucocytes, in the areas of degeneration. In area C of Fig. 1, the tubules were essentially normal.

Fig. 4 is a cross section through the center of a testis exposed for 10 minutes at 40° C to infra red irradiations. Area A, the side most directly exposed, shows coagulation of the tubules, whereas the tubules in area B show varying degrees of degeneration. The tubules farthest from the source of radiation were normal. The general picture of the degenerative changes was similar to that produced by microwave irradiation.

Discussion. Testicular degeneration resulting from exposures to microwaves and infra red irradiations presented a similar histologic appearance which was typical of the degeneration seen in experimental cryptorchidism.

The temperatures at which damage was noted from infra red irradiations were approximately 3 to 5°C lower than those reported by Moore and Chase. These investigators placed the bulb of a thermometer close to the scrotum to register the degree of heat applied. With the needle thermocouple

method of temperature measurement used in the experiments reported here it was possible to register the temperature within the testes.

Following electromagnetic irradiation testicular degeneration was found at temperatures below those at which damage occurred from infra red irradiations. All of the testes which were elevated to a temperature level of 35°C and above with microwaves were found to contain degenerated tubules.

The outcome of this experiment clearly shows that testicular damage will result from 12 cm irradiations at a temperature below that of the abdominal cavity and below that necessary to cause injury by infra red exposures. This finding suggests that damage may result in part from factors other than heat. However, it should be pointed out that measurements of temperature were made only near the center of the testes and the possibility exists that areas adjacent to the field of irradiation may have been subjected to temperatures somewhat in excess of those recorded.

These findings suggest that precautions should be taken by those working in the field of high frequency electromagnetic generators and to those giving treatments with microwave generators. Because of the unusual susceptibility of testicular tissue to thermal agents, it seems desirable to shield these structures from high frequency electromagnetic waves during periods of treatment or exposure.

Summary. A study was made concerning the effects of 12 cm electromagnetic waves and of infra red irradiations upon the testes of adult albino rats. A single ten-minute exposure to microwaves at a temperature of 35°C as measured in the central areas of the testes caused testicular degeneration in all cases. In some experiments testicular damage resulted from a single exposure at temperatures between 30° and 35°C. Testicular damage was not found in experiments in which infra red irradiation was applied for 10 minutes at 38°C but was observed when applied at a temperature of 40° C and above. The type of degeneration resulting from microwave exposure could not be distinguished from that produced by infra red.

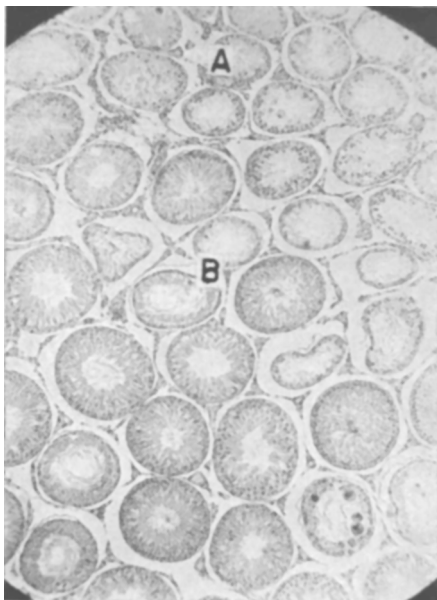


FIG. 4.

Degenerating tubules of a testis 4 days after a single irradiation with infra red at 40°C for 10 minutes. ($\times 40$).