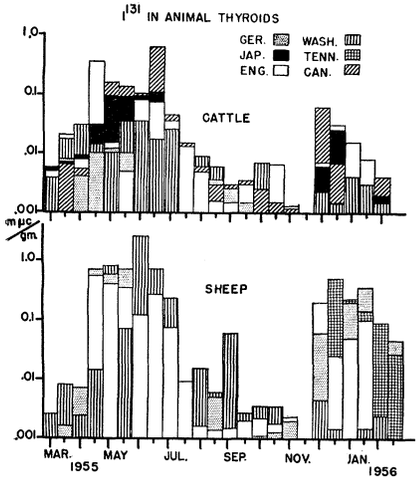

Fig. 2. Iodine-131 in cattle and sheep thyroid glands.

the last test of 15 May. The delay of 5 days to first detect radioactivity and 14–20 days to reach a maximum could suggest that a major fraction of the I^{131} fell out slowly (*2*). An additional explanation is the possibility that the environment was maximally "labeled" with I^{131} when the increase of thyroid I^{131} began. The thyroid gradually accumulated the isotope, but the I^{131} continually decayed. The result would be a rapid increase, since accumulation rate exceeded decay rate, then finally, decay rate would surpass accumulation, since the environmental intake and accumulated material both decayed.

At least the following 12 factors of unknown relative importance determined the maximum accumulation of I^{131} in thyroids distant from the test site: (i) total mass of I^{131} released, (ii) altitude of release and dust present, (iii) proportion of I^{131} or parent isotope released in gas and solid state, (iv) repeated nuclear tests, (v) distance the I^{131} traveled before detection in the thyroid gland, (vi) a flattening of the distribution curve of the radioactive concentration as the radioactive mass was diluted, (vii) weather conditions, (viii) possibility of radioactive mass returning to sample area after once passing, (ix) radioactive decay, (x) inadequacy of samples, (xi) continued intake of the isotope, (xii) rate of thyroid accumulation and release of the iodine.

The upper portion of Fig. 1 shows that I^{131} increased in human thyroid glands in Memphis when an increase was demonstrated in cattle. The maximum I^{131} per gram of human thyroid was less than 0.5 percent of the maximum for cattle from the same general area. This difference may be related to the fact that the human thyroid is more than 10 times larger than that of cattle (compared on body weight basis), but the total volume of air inhaled per day is less in the human beings. In addition, human beings do not ingest large amounts of dust in their diet.

Figure 2 shows that increases in I^{131} content of thyroids from sheep in England and Germany occurred 2 to 4 weeks after the 1 March maximum in Memphis. These delays may have been due in part to factors ii, v, vi, vii, and x. The April maximum radioactivity in the samples from England and Germany was quantitatively similar to the March maximum in the Memphis cattle.

In areas from which both sheep and cattle specimens were obtained, if the I^{131} of one species exceeded the other, the sheep were consistently the greater. This may be related to ingestion of more dust by the close-grazing sheep.

The greatest concentration of I^{131} in Memphis specimens, 1 June, did not appear to be the season's greatest concentration outside of North America. The differences between the world-wide distribution of the March and June maximum may have been related to factors i, ii, vii, and x. In the spring of 1955 the distribution of I^{131} may have been relatively uniform throughout the entire Northern Hemisphere.

Dunning (*5*) has shown theoretical methods to estimate radiation dose from radioiodine fallout. Assuming that the 1044 cattle in Fig. 1 are representative, these data make it possible to determine experimentally the radiation dose in cattle of the Memphis area. The maximum and average data of Fig. 1 were plotted on square coordinates and the curves were integrated. The result was millimicrocuries per day per gram and this was multiplied by 12.3 (mrep per day per millimicrocurie of I^{131} per gram). The data for the Memphis cattle were studied by these analyses. The November 1954 fallout delivered a maximum of 0.30 rep and an average of 0.11 rep to the cattle thyroids; the Nevada series resulted in a maximum of 13 rep and an average of 4.3 rep in cattle thyroids; the fallout in the winter of 1955–56 produced a maximum of 0.10 rep and an average of 0.04 rep in the bovine glands.

Dunning's analysis applied to the Memphis cattle data on the last test of the Nevada series (15 May) showed an average of 4 to 6 rep delivered to the cattle thyroids. This equals the 4.3 rep, average calculated here for the entire Nevada test period.

The data from England (253 glands) were the most complete of those from outside the United States, so the average radiation was calculated for cattle and sheep in England during the period April–November 1955. This estimate showed 0.15 rep and 0.40 rep per thyroid for cattle and sheep, respectively, among the specimens from England. This radiation dosage can be compared with the 5000 to 10,000 rep from I^{131} necessary to treat hyperthyroidism in human beings.

Even though I^{131} fallout was easily detected in cattle and sheep thyroids the total radiation dose to the gland was small during the period studied.

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References and Notes

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6. I wish to acknowledge the assistance of the following collaborators who sent specimens of thyroid glands from their areas: Ray B. Watkins, chief veterinarian, Seattle and King County Public Health Department, Seattle, Wash., F. D. Sowby, Department of National Health and Welfare, Ottawa, Ont., Canada; H. Miller, Sheffield National Center for Radiotherapy, Sheffield, England; Corporation of London, Metropolitan Cattle Market, London, England; Max Muhlpointner and Company K. G., Munchen, Germany; Ryoichi Tanaka, Ibaragi University, Ibaragi Ken, Japan; Memphis Packing Company (Div. of Armour & Co.), Memphis, Tenn., and the Neuhoff Packing Company (Div. of Swift & Co.), Nashville, Tenn. Human thyroids were obtained through the courtesy of M. L. Trumbull, pathology department, Baptist Memorial Hospital, Memphis, Tenn. The survey of USPH monitors was made possible through the help of R. A. Dudley of the division of biology and medicine, U.S. Atomic Energy Commission, and the cooperation of O. Placak and C. Powell of the USPHS.
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Gum Replica Technique for Electron or Light Microscopy

In a study of the effects of organic solvents on the surface structures of plant-rust spores, the need arose for use of replica techniques in order that surface changes on the spore could be observed with the electron microscope. Replica techniques involving the use of heat, pressure, or organic solvents could not be utilized because of the sensitivity of spores to these treatments. Attempts to make replica patterns of rust spores by evaporation of silicon monoxide, carbon [D. E. Bradley, *Brit. J. Appl. Phys.* 5, 65 (1954)], and similar substances were unsuccessful, because the relatively large

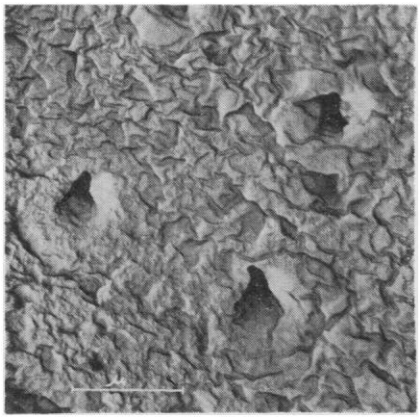

Fig. 1. Two-stage replica of a spore of wheat-stem rust that was obtained by using gum acacia and collodion. The replica is shadowed with uranium at a slope of 1: 2. The surface is covered with wrinkles that are as high as 0.2 μ. Spines averaging 0.5 μ in height are spaced at intervals of about 2 μ. The size and shape of these spines may be verified by examination of the periphery of the spore itself in the electron microscope.

size of the spores (15 by 30μ) prevented the formation of an even layer with the necessary thickness and strength. When the specimen was rotated through 360° during evaporation, the afore-mentioned replicating materials enveloped the particles and prevented their removal from the film.

The usual two-step replica process using gelatin or methyl cellulose in water for the negative, and collodion for the positive, replica was generally not successful. The primary reason for this difficulty is the absence of control over the depth of the negative replica. For instance, if rust spores, which are usually elliptical in cross section, are allowed to sink to a depth greater than one-half of the minor axis, the opening, through which the spore must pass if it is to be removed, will have a diameter smaller than the length of the major axis and prevent removal of the spore from the replica material. If the replica technique is to be consistently successful, the depth of the negative replica or impression must be controllable within narrow limits. It was this requirement that led us to develop a replica technique that employs gum acacia. It has proved successful for spores of *Bacillus subtilis* and stem rust of wheat. The technique may be described as follows.

1) A saturated solution of gum acacia in water, containing a small amount of formalin to retard spoilage, is filtered through a medium sintered-glass filter or its equivalent.

2) A glass slide is coated by being dipped into the gum solution. Care must be taken to prevent the formation of bubbles on the surface of the slide in this procedure. The slide is then dried in a vacuum desiccator. Slides may be kept indefinitely and used as needed for routine samples.

3) The dry specimen is spread evenly over the gum surface, and the excess is shaken off by tapping the slide.

4) The slide is held face-down over warm water until a thin layer of gum is liquefied by the moisture. Both the temperature of the water and the exposure time may be adjusted to modify the extent to which the specimen penetrates the gum. Fifteen seconds' exposure 1 in. above water at 50°C gave good results with rust spores, while the best exposure time for bacterial spores was found to be 5 seconds. In each case only a small, relatively flat portion of the surface of the specimen formed an impression.

5) The slide is dried thoroughly in a desiccator, after which the specimen is brushed from it with cotton or cheesecloth. The hard surface of the gum is not damaged by this treatment, and the cloth does not actually strike the negative replica surface, which is visible as a depression with the light microscope. Examination at suitable magnification will indicate whether an adequate negative impression has been made and whether the specimen has been removed from the impression.

6) The slide with the negative gum replica is immersed in 1 percent collodion in amyl acetate and dried. The collodion is scored into small squares with a needle, and the slide is immersed in water to dissolve the gum and free the collodion. The positive collodion replica is caught face-up on a 200-mesh specimen screen, shadowed with uranium, and examined in the microscope.

Removal of the specimen from the gum slide by brushing is practical in the formation of replicas of particulate matter larger than about 0.5μ in diameter. Smaller objects of nonbiological materials may be removed by being dissolved in a suitable solvent that will not etch or distort the gum. The gum technique has been the only means by which we have been able to make a replica of plant rust spores or bacterial spores without the disadvantages brought about by techniques that employ heat, pressure, or organic solvents. The gum was found to yield replicas of polystyrene latex particles with no demonstrable distortion. A resolution of better than 200 A was obtained. Figure 1 shows a positive replica of a spore of wheat-stem rust and indicates the surface detail that the method is capable of reproducing.

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Action of p-{Di(2-chloroethyl)}-amino-L-phenylalanine on Harding-Passey Mouse Melanoma

Bergel and Stock have reported (*1*) an almost complete carcinostasis against the Walker rat carcinoma as a result of injecting *p*-{di(2-chloroethyl)}-amino-L-phenylalanine 1 day after implantation of the tumor. It seemed of interest to investigate the activity of this compound against mouse melanoma, partly because of the known resistance of the melanomas toward mustards, other chemotherapeutic substances, and x-rays (*2*). It will also be noted that the compound in question is a derivative of phenylalanine—an amino acid that serves as the ultimate precursor of the melanin that is so actively deposited by the melanocyte. From what is known of the phenomenon of metabolic antagonism, investigation of a phenylalanine derivative as a possible cytostatic agent against melanoma would seem to be worth while, even though the opinion is widely held that melanin formation is independent of the basic and vexing problem of tumor growth.

The experiments reported here were carried out on the Harding-Passey melanoma in dba, line 1, mice (*3*). The compound investigated was synthesized by the method of Bergel and Stock (*4, 5*). Resolution was achieved through the brucine salt of N-acetyl-*p*-nitro-DL-phenylalanine, an advanced intermediate in the synthesis.

Thirty female dba mice, 7 to 8 weeks of age, were implanted on 8 October with the Harding-Passey melanoma from donor mice originally implanted 3 weeks earlier. The mice, then weighing about 14 g each, were divided by random selection into three groups, A, B, and C, of ten each.

On 9 October the mice of group A were injected intraperitoneally with the nitrogen mustard suspended in peanut oil. Each animal received 0.4 mg in 0.4 ml of peanut oil. By the fifth day it became apparent, despite the indications of preceding toxicity experiments on other mice, that this was virtually a lethal dose.

On 17 October the mice of group B were beginning to show very small tumors that could be discerned by palpation. On this date (9 days after implantation) each mouse was injected intraperitoneally with 0.2 mg of the nitrogen mustard suspended in 0.4 ml of peanut oil (*6*).

On 1 November six mice selected at random from group B (all ten were alive) and six from group C were sacrificed. The tumors were excised and weighed. The tumors of the B group were uniformly small and weighed on the average 0.028 g each; those of the C mice were slightly more variable in