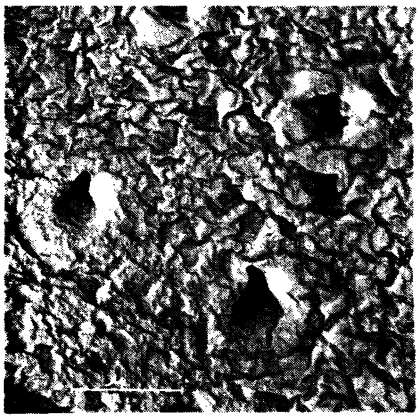

Fig. 1. Two-stage replica of a spore of wheat-stem rust that was obtained by using gum acacia and collodion. The replica is shadowed with uranium at a slope of 1: 2. The surface is covered with wrinkles that are as high as 0.2 μ. Spines averaging 0.5 μ in height are spaced at intervals of about 2 μ. 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

size but, in all cases, were much larger than the B-mouse tumors and weighed an average of 0.678 g each.

The mice of group B, with average tumor weights of 0.028 g when they were sacrificed, weighed, on the average, 14.3 g on implantation, 16.4 g on the fifth day (22 October) after injection of nitrogen mustard, and 17.5 g on the day before they were sacrificed (31 October). The control mice of group C, with average tumor weights of 0.678 g when they were sacrificed, weighed, on the average, 14.3, 17.0, and 17.7 g on 8 October, 22 October, and 31 October, respectively.

Although the findings strongly suggest that the single dose of 0.2 mg of the nitrogen mustard achieved a complete cessation of growth of the implant, experiments have yet to be performed to determine whether actual regression of a tumor of appreciable size can be achieved and whether the small static tumors of the B mice are viable.

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References and Notes

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3. Roscoe B. Jackson Laboratories, Bar Harbor, Maine.
4. F. Bergel and J. A. Stock, *J. Chem. Soc.* 2409 (1954); F. Bergel, V. C. E. Burnop, J. A. Stock, *J. Chem. Soc.* 1223 (1955).
5. I am indebted to F. Bergel and J. A. Stock for their kindness in providing reference samples of the DL-, D-, and L-forms of the compound and to Howard Smith for synthesizing quantities of the DL-mixture and the L-isomer. Sally Williams and John Zemp assisted with the implantations and the injections. This work was carried out with assistance from the Cancer Institutional Grant, administered by Stanford University on behalf of the American Cancer Society.
6. This dosage approximates 14 mg/kg body weight.

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Increased Frequency of Births in the Morning Hours

It is generally felt that the incidences of day and night births are practically identical. Supporting this feeling is Guthmann's paper on the subject in which he combined 26,707 cases of his own with 95,087 culled from earlier literature (*1*). The resulting 121,794 cases showed that 49.1 percent of the births occurred during the day (6 A.M. to 6 P.M.) and 50.9 percent at night (6 P.M. to 6 A.M.).

The results reported here show a similar ratio between day and night—49.9 and 50.1 percent, respectively, among 33,215 births; but such an artificial division of the day into two 12-hour periods distorts the picture entirely. By dividing the day into hourly periods, and tabulating the births for each hour, one finds a very marked and statistically significant peak in the morning hours. Using his own cases, Guthmann also found a peak in the morning hours, but this finding seems to have been neglected.

Excluding Caesarian section, second or third twins, and mid- and high-forceps delivery, human births at five hospitals in three different cities were classified by time of delivery. There were 33,215 such births: 8760 from the Edward W. Sparrow Hospital, Lansing, Mich., during a 3-year period; 2532 from the St. Lawrence Hospital, Lansing, during a 1-year period; 10,546 from the W. C. A. Hospital, Jamestown, N.Y., during an 8-year period; 9421 from the Jamestown General Hospital during a 9-year period; and 1956 from the Warren (Pennsylvania) General Hospital during a 2½-year period.

The 8 consecutive hours of greatest frequency of birth, the 8 consecutive hours of lowest frequency of birth, and the 8 remaining hours were compared with one another. Thus, three statistical populations were established, and the means were compared. Such comparison was made for each of the five hospitals individually, and for the total.

Combining the data from all five hospitals (Table 1) and using the formulas

$$\frac{\text{S.D.}}{\sqrt{8}} = \text{Standard deviation of mean} \quad (1)$$

and

$$\text{S.D. of difference between means} = \sqrt{(\text{S.D. mean}_1)^2 + (\text{S.D. mean}_2)^2} \quad (2)$$

gave the following results. (i) There was a mean of 1561 births (S.D. 47) for each hour during the peak hours of 3 A.M. to 11 A.M. (ii) There was a mean of 1213 births (S.D. 66) for each hour during the low hours of 3 P.M. to 11 P.M. (iii) There was a mean of 1375 births (S.D. 65) for each hour during the remaining hours of 11 A.M. to 3 P.M. and 11 P.M. to 3 A.M. (iv) During the peak hours, 28.7 percent more births occurred than during the low hours. The standard error of the difference of the means of the two groups was 28; hence the difference is extremely significant statistically (12 σ), the odds being 1 in approximately 6×10^{32} that this difference is due only to chance. By taking three standard deviations of the difference between the means of the peak and low hours, one obtains a meaningful range of 21 to 36.9 percent. (v) The peak hour was 5 A.M.; it showed 48 percent more births than the low hour of 7 P.M.

Analysis of the data from each hospital individually was even more striking than the analysis of the total. Each of the five hospitals showed a statistically significant difference between the means of the 3 A.M.-to-11 A.M. and the 3 P.M.-to-11 P.M. periods. The number of standard deviations in each group is as follows: (i) Sparrow Hospital, 11 σ; (ii) St. Lawrence Hospital, 4.5 σ; (iii) Warren General Hospital, 3.25 σ; (iv) W. C. A. Hospital, 7 σ; (v) Jamestown General Hospital, 10 σ.

Table 1. Number of births each hour of the day in five hospitals.

Time	No. of births
Group A (3 A.M. to 11 A.M.)	
3	1590
4	1560
5	1632
6	1547
7	1470
8	1588
9	1585
10	1515
Group B (11 A.M. to 3 P.M and 11 P.M. to 3 A.M.)	
11	1422
12	1418
1	1480
2	1416
11	1355
12	1297
1	1281
2	1335
Group C (3 P.M. to 11 P.M.)	
3	1134
4	1276
5	1180
6	1213
7	1103
8	1267
9	1298
10	1253

By taking the odds in each of these five and multiplying them together, one can estimate that the likelihood of the differences between the peak and low groups being due to chance is 1 in about 10^{69}.

I did a similar analysis of Guthmann's 26,707 cases and found that the period from 2 A.M. to 10 A.M. showed 7.8 percent more births than the period from 2 P.M. to 10 P.M. This difference was statistically significant (4 σ) and, although it is not great, it tends to corroborate the findings presented here. The periods are not identical with mine because Guthmann's cases were tabulated in periods of 2 hours.

To argue that there are "only 33,215" cases here and hence not enough to prove the point is to ignore the fact that each of the five hospitals alone showed similar peak and low hours that were statistically significant. The same results in many smaller groups are more significant than a certain result in one large group.

It may be felt that the inclusion of low-forceps deliveries disguises the true situation. However retabulation of the 10,546 W. C. A. Hospital cases showed the same