Bacillus subtilis grows luxuriantly in this medium, there being approximately 200,000,000 cells present per cubic centimeter of the culture after 48 hours' incubation at 37 degrees Centigrade. Sixty to 70 per cent. spores are regularly present within five days (100 per cent. in the pellicle). With Parke-Davis peptone in a 1 per cent. solution as a control, there were 370,000,000 cells, but less than 30 per cent. of these were as spores.

The simplicity of the medium and its capacity for supporting good growth and the rapid production of a high percentage of spores by *B. subtilis* justifies its use in future spore studies with this organism.

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A SIMPLIFIED TECHNIQUE FOR MOUNTING GROUND BONE SECTIONS TO SHOW AIR-INJECTED LACUNAE AND CANALICULI

THE usual method for such sections is to place a small lump of solid balsam or solidified 10 per cent. gelatine on both slide and cover-glass. Apply enough heat to dissolve the mounting medium, place bone section in liquefied balsam or gelatine, cover and cool rapidly.

Better results are obtained by mounting the dry bone section in either thin or medium thick damar balsam and permitting the balsam to dry in air. No cover-glass should be used. The specimen may be examined while the balsam is drying, for the canaliculi and lacunae become evident immediately and are much more outstanding than specimens in which the bone spaces are filled with débris.

Bone sections so prepared keep indefinitely and are much more satisfactory than those in which heat and cover-glass are necessary. By employing the technique for grinding bone already described in SCIENCE, Vol. 75, No. 1945, "Aloxite as an Abrasive for Grinding Bone Sections for Histology," in conjunction with this simplified mounting technique, an ideal method for preparation of bone sections for classroom use is achieved.

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SPECIAL ARTICLES

X-RAY DIFFRACTION STUDIES OF THE BUREAU OF STANDARDS RUBBER FRACTIONS

AT the request of Dr. Washburn, a careful preliminary x-ray diffraction study has been completed on various samples of rubber fractions prepared at the Bureau of Standards by the methods already described.[1] The results are so interesting, especially in structural differentiation between the ether-soluble (sol-rubber) and the ether-insoluble (gel-rubber) hydrocarbons, and in producing new data bearing upon the several theories of the structure of whole rubber, that it has seemed desirable to record very briefly some of the more important observations. It will be recalled that native rubber, raw, purified or vulcanized, gives a typical liquid halo or "amorphous" type of diffraction pattern in the unstretched condition, which changes to the characteristic crystal fiber pattern when the rubber specimen is stretched. Upon the basis of these facts several theories of the structure of the rubber hydrocarbon have been proposed: the folded or spiral molecules model; the fringe model (bundles of molecular chains with frayed ends); the flexible chain model and the very familiar two-phase model.

The important new observations on the fractions are as follows: (1) The purified total hydrocarbon behaves on stretching exactly as previously found for rubber.

(2) *The sol-rubber fraction produces no evidence whatever of the characteristic crystal fiber pattern upon stretching even up to 1000 per cent.* The liquid halo is retained under all conditions, but it becomes *broader* in proportion as the percentage of stretch increases (which may be due to decreasing particle size). The same results are observed in the presence or absence of antioxidants.

(3) The gel-rubber fraction produces easily, above 100 per cent. elongation, the crystal fiber pattern, which is quite sharp and intense for 200 per cent. stretch. The liquid halo remains unchanged in width and decreases in intensity as the crystal interferences increase in intensity. When, however, antioxidants are removed and the sheet allowed to stand without vulcanization, it is difficult to find crystal interferences even at 400 per cent. elongation.

(4) The sol-rubber *when vulcanized* begins to show faint evidence of crystal interferences when stretched above 400 per cent., showing that sulfur has produced a profound structural effect. Vulcanization was accomplished by the Peachey (vapor cure) method.

(5) The gel-rubber, vulcanized, gives a fiber pattern at 250 per cent. elongation.

In a detailed paper there will be presented:

Quantitative measurements on 50 or more diffraction films, correlation of these structural observations

[1] Washburn, *Phys. Rev.*, 38, 1790 (1931); Smith, Saylor and Wing, *Bureau of Standards Journal of Research*, 10, 479 (1933).

with physical data such as stress-strain curves, a critical comparison of the theories of rubber hydrocarbon structure in the light of these results, and further x-ray studies of the rubber hydrocarbon crystals actually produced for these fractions at the Bureau of Standards.

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EFFECTS OF ENDOCRINE EXTRACTS ON THE EARLY DEVELOPMENT OF THE CHICK

THIS study was begun in the fall of 1930 as an attempt to analyze the effect of various endocrine extracts on the morphology of certain organs in the chick embryo and to see if it might be possible to bring about sex reversal. With a greater perfection of technique the studies are taking a decided trend toward sex reversal.

Two methods have been used in this work, the first of which was to inject extract directly into the allantois through the shell. This procedure has several disadvantages: the amount of material injected must be small, a piece of shell must be removed or the injections are not easily made until after about the sixth day of incubation, and lastly, the danger of infection is rather great.

The second method, and the one being used now, is essentially the one described by Hanan, Atwell and Hurd in their researches on the absorption of vital dyes by the allantois. These workers were able to show that in a large percentage of the cases trypan blue could be absorbed by the allantois from the air chamber of the egg as early as the fourth day of incubation. As much as 0.5 cc can be injected at that time. Their technique has been modified very little and it has been found that it is possible to inject twice daily over a period of days. By using the air chamber the amount of extract injected can be regulated better, the danger of infection is reduced to an insignificant factor and injections are made on the fourth day, which is approximately two days before the histological differentiation of the gonads.

A number of endocrine extracts have been used, but most of the experiments have been performed with Emmenin, Whole Pituitary, Antuitrin-S and Theelin. These have been furnished me through the kindness of Eli Lilly and Company and Parke, Davis and Company. The recent work has been limited almost entirely to the use of Antuitrin-S and Theelin. The major difficulty encountered to date has been the regulation of dosage. This obstacle led to a large number of fatalities in the early work, but it has been remedied in the later experiments.

The following results are presented as being indicative, but not with the idea that they represent in any sense completed work. Since it is not possible to analyze the experiments as separate units here, the results are given in summary.

Five hundred and sixty-eight embryos have recently been examined after having been treated with the extracts mentioned above, with the following results: 305 ♀ ♀; 170 ♂ ♂; and 93 unknown. The 93 unknown embryos died before sex could be determined. The percentage ratio is 64.3 per cent. females to 35.7 per cent. males. No attempt was made at selection of data. Both the earlier and later experiments were used in calculating these percentages, and as a result the number of unknown cases is high. However, even if all the 93 unknown ones are considered as males, the percentage of females over males would be 7.5 per cent., or 42 embryos.

If, on the other hand, the later experiments be considered as a unit, these having a much lower percentage of unknown cases, we find the following figures: 146 ♀ ♀; 69 ♂ ♂; and 15 unknown. If again we consider all the unknown as males, the number of females still exceeds the males by 62, or 27 per cent. Since all the extracts have fractions which affect the female reproductive system, the results appear significant. Also, there have been no experiments in which the ratios have not favored the females. Some male embryos have had asymmetrical testes. Accordingly, questionable gonads have been studied from histological preparations in order to prevent the possibility of interpreting these as ovaries. An interesting thing which has occurred is that the early fatalities, which come after sex can be determined, have been predominately of female embryos, running as high as 83 per cent. in the recent experiments. The experiments with Theelin offer additional evidence against the possibility of all the early deaths being of male embryos. To illustrate, the first three Theelin experiments are examples:

Experiment	I	25 ♀ ♀; 14 ♂ ♂;	11 unknown.
Experiment	II	24 ♀ ♀; 13 ♂ ♂;	13 unknown.
Experiment	III	34 ♀ ♀; 14 ♂ ♂;	2 unknown.

As can be seen from the figures, the dosage was regulated best in Experiment III, and it would appear that the preponderance of females was increased by the addition of members constituting the unknown class in Experiments I and II. The Antuitrin series give similar results.

A large number of eggs have been injected with