

chromatographed, certain sections of the column or the filtrate showed fluorescence in ultraviolet light.

It was now found that some of these faintly colored (or colorless) substances can be separated and accumulated in well-defined zones on a calcium hydroxide column. Among others one of the compounds mentioned which shows an intense, somewhat greenish fluorescence in hexane or alcohol solution possesses a characteristic spectral curve. In hexane three sharp maxima appear in the near ultra-violet region, *viz.*, at 367, 348 and 332  $m\mu$  (Fig. 1). Similar is the extinction curve of the alcohol solution, with sharp maxima at 368, 349 and 332-3  $m\mu$ . The

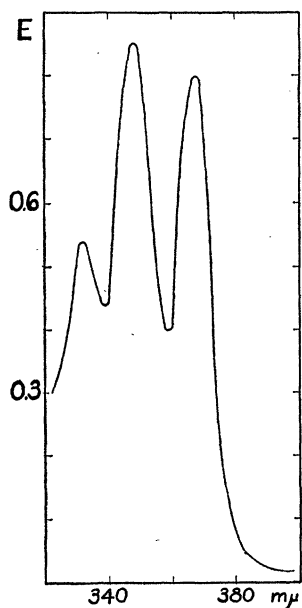


FIG. 1. Extinction curve in hexane. (Fluorescing compound *ex* tomatoes).

highest maximum is located at about 20  $m\mu$  longer wave-length than that of vitamin A and differs by a few millimicrons only from that of vitamin A<sub>2</sub>.

The curve as represented in Fig. 1 shows a fine structure in the fundamental band, in contrast to the corresponding curves of the two vitamins mentioned. It is, however, very similar to the extinction curve<sup>3</sup>

of anhydro vitamin A ("cyclized" vitamin A),<sup>4</sup> the alcoholic solution of which shows maxima at 392, 371 and 351  $m\mu$ . Nearly identical in wave-lengths are the maxima of "iso-anhydro vitamin A" (about 370, 350 and 330  $m\mu$ )<sup>3</sup> and of our compound.

The compound under discussion is epiphasic when partitioned between hexane and 90 per cent. methanol. In the mixed-chromatogram test it is adsorbed much below the vitamin A, a little below the  $\beta$ -carotene and in the region of the  $\alpha$ -carotene zone. The polyene structure of this compound follows from the nature of its spectrum, from the positive Carr-Price reaction and from its behavior toward iodine. When a hexane solution is catalyzed with iodine and illuminated at room temperature, a subsequent chromatogram shows substantial amounts of isomerization products. Much less is the extent of spontaneous isomerization. The isomers form well-defined zones below the unchanged portion on the Tswett column which fluoresce in ultraviolet light.

Our compound seems to be widespread in nature, since its extinction curve was frequently observed with extract fractions of very different starting materials. It was first obtained when some flowers, belonging to various families, were investigated, *viz.*, *Bignonia* sp., *Tecomaria capensis*, *Gelsemium sempervirens* and *Gazania rigens*. The green leaves of wild oat (*Avena* sp.) tested contained only a very small quantity of the compound. The latter could possibly play some part in our nutrition, since it occurs in relatively marked amounts in carrots as well as in fresh or canned tomatoes, and in orange pulp and peel.

To the authors' knowledge well-characterized polyenes of the indicated type have hitherto not been found in the vegetable kingdom. However, it is possible that a fraction of our vitamin A supply may originate from certain plant products whose molecules contain a much shorter conjugated system than the carotene chromophore.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A 100 KV ELECTRON MICROSCOPE

In the first few years of electron microscopy, development and research was entirely carried out by uni-

<sup>3</sup> E. M. Shantz, J. D. Cawley and N. D. Embree, *Jour. Am. Chem. Soc.*, 65: 901, 1943.

versity laboratories. Soon, however, the commercial value of this new instrument was discovered, and the industrial development started, first abroad and then

<sup>4</sup> J. R. Edisbury, A. E. Gillam, I. M. Heilbron and R. A. Morton, *Biochem. Jour.*, 26: 1164, 1932.

in this country too. Very valuable work was done by those industries and for a while it looked as if very little was left over for academic research. Also it seemed as if that little could not be efficiently tackled, if the means, put at the disposal of industrial and academic organizations, were compared.

However, it soon became apparent in the field of electron microscopy, as in other branches of science which were industrially developed, that continuation of academic research is not only valuable, but also necessary. One of the first institutions, or perhaps the first, to recognize this fact and to start a research program of its own in this field was Stanford University. As part of this research program an electron microscope was designed and constructed which shows in several respects departures from known instruments. A brief description of this first Stanford electron microscope seems therefore justified.

The word "universal" has been used before for characterizing two electron microscope designs. If this word is used to mean a versatility of the instrument and its adaptability to different types of observations, then the Stanford microscope can truly be designated as a universal electron microscope. It is a three-stage compound microscope for bright-field and dark-field illumination, for wide-angle stereoscopy, and is provided with means for conversion into a diffraction camera. It has a number of other features which will be mentioned below in a little more detail. Its voltage range is from 30,000 volts up to 100,000 volts in steps of 10,000 volts.

Three-stage magnification was chosen for this instrument because it offers the advantage of observing a wider field at lower magnifications than a two-stage microscope is capable of. Also due to the wide field at our disposal at low magnifications, the necessity of observing the first stage (intermediate) image is eliminated and the construction of the microscope simplified. The optical arrangement differs from the conventional electron microscope inasmuch as the alignment on the optical axis is achieved by means of moving the pole pieces of the magnetic lens instead of the whole coil. The instrument being provided with a fluorescent screen for "end-on" observation, the optical system of the microscope is easily accessible from both ends of the instrument for cleaning or any necessary changes.

The object chamber of the microscope is distinguished from other similar constructions by the greatly reduced volume of its air lock and by the peculiar mechanism of its stage. The stage is suspended on a bracket, provided for a frictionless cross-movement when scanning the surface of the specimen. The cross-movement of the stage is operated from the posi-

tion of the observer through a hydraulic transmission built with a coarse and fine adjustment. The coarse adjustment is a great help when first scanning at low magnifications. The fine adjustment of the stage can be adjusted with an accuracy of a few hundred Angstrom units. The supporting bracket of the stage can be tilted by means of a handle placed outside the microscope and thus the stage is tilted up to  $\pm 15\frac{1}{2}^\circ$ .

The photographic chamber of the microscope is of the magazine type. Up to 24 plates can be stacked up in its magazine, separated from the microscope by a gate. The plates can be individually exposed in such a manner that either full or partial exposures are obtained. They can be taken out individually from the microscope without impairing the vacuum, thus assuring an uninterrupted operation. Observation of the image is generally carried out on a fluorescent screen above the photographic plate. A second transparent fluorescent screen is provided, however, at the bottom of the instrument which allows viewing of the image by a greater group of observers than by the individual portholes. Another advantage of this "end-on" screen is that it can easily be removed. Quite recently extensive use has been made of this facility during the development of an electron microspectroscope for the analysis of minute specimens.

It was found convenient to develop a new type of specimen holder for this microscope. Originally flat discs provided with center holes were used for supporting the specimens or the thin membranes used as "slides."<sup>1</sup> They were later replaced by wire meshes cut to a circular disc of convenient diameter.<sup>2</sup> In the new design wire screens are maintained as the supporting element; but, instead of being flat, they are cup-shaped and inserted in small commercial eyelets. There is a flat surface of 1 mm diameter, perpendicular to the optical axis, which is the observed part. The advantage of this type of specimen holder is that it is easy to handle, it is "polarized," *i.e.*, there can not be any confusion between front and back surface, and furthermore, it is easy to file for further use. The "cartridges" or supports of other electron microscopes can easily be modified for the use of this new type of specimen holder.

Following the practice, which becomes more and more accepted, of caution in stating the resolving power of the instrument, it will be mentioned that the instrument described herein has a resolving power of better than 50 Å.U. It has been used up to now for a series of investigations from which the following might be mentioned: ferromagnetic domains, poliomyelitis virus, different bacteria and their modifica-

<sup>1</sup> Ruska, 1934, Marton, 1934.

<sup>2</sup> Marton, 1940.

tion under action of physical, chemical or biological agents, etc. The detailed account of these investigations will be published elsewhere.

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### A WORKING MODEL OF THE HUMAN CIRCULATION

THE circulation schema here pictured and described has proved helpful in explaining the complexities of the circulation to beginners in physiology. Its construction is simple and inexpensive<sup>1</sup> and its relation to the human body is much more direct than that of commercial models.

Most of the essential features are visible in the illustration. The heart consists of four rubber bulbs operated by cross-bars attached to a central rod, the sequence of events being as in the heart itself. Small

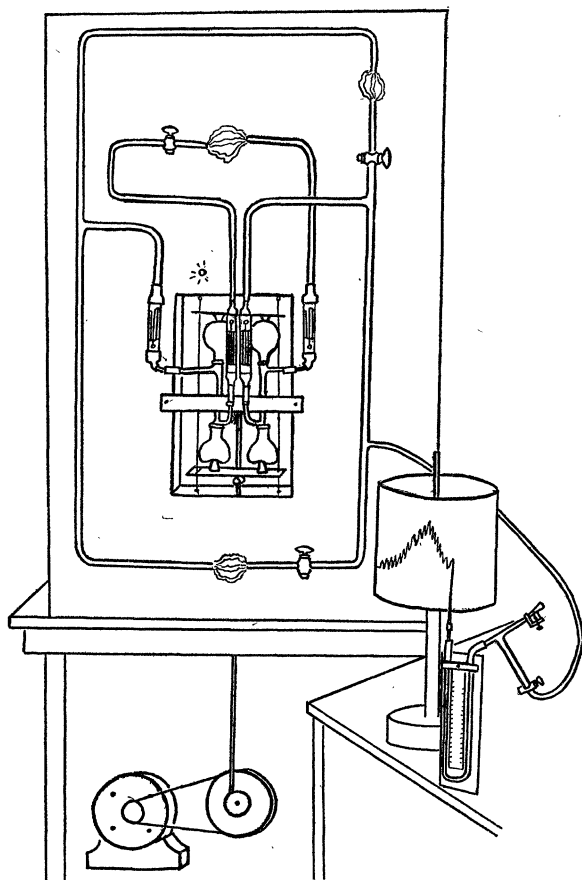


FIG. 1. This drawing shows how the effects of a simulated injection of adrenalin may be demonstrated. The motor has been speeded up and the stop-cocks in the systemic circulation narrowed.

<sup>1</sup> The device may be purchased from the Denver Fire Clay Company.

light bulbs flash on at the proper times to represent the activation of the S. A. and A. V. nodes. The connection of blood vessels with heart is through one-way valves, glass flutter valves being employed which can be seen in operation. Glass tubing is used for blood vessels, the fluid being appropriately colored, methylene blue for venous and Congo Red for arterial blood. Peripheral resistance is obtained by stop-cocks representing arterioles; in the systemic circulation one serves muscle tissue, the other is located in the splanchnic region. Anatomic regions of the body are painted in lightly, as background, on the panel. Beyond the arterioles a capillary network is indicated and by a proper arrangement of the tubes the emerging blood has the opposite color of that entering, *i.e.*, changes from blue to red in the pulmonary, and from red to blue in the systemic circulation. The aorta is cannulated and connected to a manometer writing on a kymograph drum. Heart sounds are reproduced by electrical contacts and a loud speaker.

Among the physiologic events which can readily be demonstrated are the sequence of events in the cardiac cycle, the details of the circulation and the effect of various factors on the blood pressure, such as alterations in the cardiac output, variations in the peripheral resistance, the shunting of blood from visceral regions to muscles during exercise, the loss of blood in hemorrhage, etc.

It is evident that, except for motor, kymograph and loud speaker, no expensive items are required. The motor can be dispensed with, manual operation being satisfactory, a kymograph is available in most laboratories and a loud speaker can be found in almost any basement.

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