inferred from certain radiocarbon dates previously obtained from the Valley of Mexico (*4*). The charcoal from the Rio Hondo gravel at Tlatilco gave an average date of 6390 ± 300 years before the present for the Totolzingo phase. The date for the mammoth site at Santa Isabel Iztapan, near Tepexpan, as previously cited, is identical with one furnished last year by the Division of Geochemistry of the U.S. Geological Survey for the matrix of a giant fossil armadillo from the Upper Becerra formation (*5*). Its fauna may therefore have become extinct between 9000 and 8000 years ago. In view of the stratigraphic position of the burial at San Vicente Chicoloapan, its age may be 8000 to 6000 years.

The artifacts found so far have been deposited in the Direccíon de Prehistoria, which will continue the excavation (*6*).

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3. The latter date was supplied by J. L. Kulp of the Lamont Geological Observatory of Columbia University.
4. H. de Terra, "Comments on radiocarbon dates from Mexico," in F. Johnson, "Radiocarbon dating," *Am. Antiquity* 12, No. 1 (1951).
5. Information supplied by Ing. A. R. V. Arelleno of the Instituto Nacional de Geología in Mexico City.
6. Previous to my summer visit in Mexico I had the pleasure to attend, at the end of February, the opening of the Museo de Prehistoria at Tepexpan. Its simple but very attractive modern design provides a large hall, in the center of which is the original site of Tepexpan Man, surrounded by exhibition show cases, wall charts, and photographs. Situated on the road to the pyramids of Teotihuacan, a site frequented by thousands of visitors, it symbolizes the start of prehistoric studies in Mexico and the progress of public education in that country. The opening was among others attended by those Mexican colleagues to whom I feel deeply obliged for their generous aid and cooperation, notably Dr. Eusebio Davalos, professors P. Martinez del Rio and Arturo Romano P., and Luis Aveleyra A. de Anda.

4 November 1958

Antigenicity of Steroid-Protein Conjugates

Abstract. Testosterone, cortisone, deoxycorticosterone, estrone, and progesterone act as haptens when they are conjugated with bovine serum albumin. Antibodies with steroid specificity are formed in rabbits immunized with each of the five steroid hormone–protein conjugates.

Five steroid hormones, testosterone (T), cortisone (C), deoxycorticosterone (D), estrone (E), and progesterone (P), were coupled to bovine serum albumin (BSA) (Armour, fraction V). Six conjugates were formed, two containing testosterone, and one containing each of the other steroids. The conjugates are referred to as T-3-BSA, T-17-BSA, C-21-BSA, and so forth, the number indicating the carbon atom through which the steroid moiety was linked to the protein. The synthesis and chemical properties of T-3-BSA, T-17-BSA, and C-21-BSA have been described in detail (*1*). E-17-BSA, P-20-BSA, and T-3-BSA were prepared by use of the O-(carboxymethyl)-oxime. D-21-BSA was synthesized by use of succinate in a manner analogous to that used in the preparation of C-21-BSA.

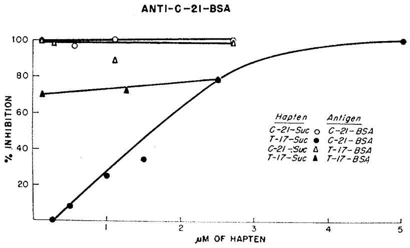

Fig. 1. The inhibition by cortisone-21-succinate and testosterone-17-succinate of precipitation of anti-C-21-BSA with C-21-BSA and T-17-BSA.

Antisera were obtained from rabbits immunized with alum-precipitates of each of the steroid-protein conjugates (*2*). All antisera precipitated with BSA as well as with the conjugate used for immunization. The antisera therefore were absorbed with BSA before use to eliminate that portion of the reactions between the conjugates and the antisera dependent on residual BSA specificity of the conjugates. Quantitative antibody determinations followed the procedure described by Kabat and Mayer (*2*), in which the Folin-Ciocalteu method (*3*) for analysis of the specific precipitates is used.

After absorption with BSA, it was found not only that the antisera react with the steroid-protein conjugate used for immunization, but also that cross-reactions among the steroid-protein conjugates also occurred. For example, at the respective points of maximal precipitation, D-21-BSA and P-20-BSA precipitated 67 percent, and T-3-BSA precipitated 50 percent, of the antibody from an anti-T-17-BSA serum precipitable by T-17-BSA.

The hapten-inhibition technique (*4*) has been used to demonstrate the steroid specificity of the antisera. In these studies, the steroid itself, as the hemisuccinate or the O-(carboxymethyl)oxime, was incubated with the antiserum prior to the addition of the homologous antigen. The effectiveness of each hapten in inhibiting precipitation could be calculated by comparing the precipitate formed in the presence of the hapten with the precipitate formed without the hapten. The results shown in Table 1 indicate that each antigen-antibody system was inhibited by the homologous hapten and so demonstrate the steroid-specificity of the antisera.

The specificity of the reaction between antigen and antibody is also indicated from a comparison of the inhibiting effectiveness of T-17-hemisuccinate with the homologous hapten. As is shown in Table 1, for each system where this has been done, the homologous hapten is a better inhibitor than T-17-hemisuccinate.

The hapten-inhibition technique may also be used to elucidate the chemical basis for the specificity of the antisteroid sera by determination of the inhibitory effectiveness of a large number of haptens. The results of some experiments designed to determine the specificity of anti-T-17-BSA are recorded in Table 2. As with the other antisera, the homologous hapten, in this case T-17-hemisuccinate, was a better inhibitor than any of the other haptens studied. Compound S-21-succinate, which differs from T-17-succinate by possessing a dihydroxy side

Table 1. Inhibition of the reactions between the steroid-protein conjugates and their homologous antisera by the homologous hapten and testosterone-17-succinate. Suc, succinate, OCMO, O-(carboxymethyl)oxime.

Antiserum	Hapten	Amt. (mg)	Inhibition (%)
T-17-BSA	T-17-Suc	0.1	74
T- 3-BSA	T-3-OCMO	0.1	58
T- 3-BSA	T-17-Suc	0.5	25
C-21-BSA	C-21-Suc	0.05	93
C-21-BSA	T-17-Suc	0.5	55
D-21-BSA	D-21-Suc	0.01	90
D-21-BSA	T-17-Suc	0.1	80
P-20-BSA	P-20-BSA	0.005	80
E-17-BSA	E-17-OCMO	0.1	96
E-17-BSA	T-17-Suc	0.5	77

Table 2. Inhibition of the reaction between T-17-BSA and anti-T-17-BSA by various soluble steroid derivatives. Suc, succinate; OCMO, O-(carboxymethyl) oxime.

Hapten	Amt. (mg)	Inhibition (%)
T-17-Suc	0.1	74
Compd. S-21-Suc	0.1	66
C-21-Suc	0.2	63
Hydrocortisone-21-Suc	0.1	36
T-3-OCMO	0.1	12
E-17-OCMO	0.2	4
Estradiol-17-Suc	0.2	0

chain at C-17, is almost as effective an inhibitor. The effect of substitution at the 11-position depends upon the nature of the oxygen function. The presence of a ketone group at C-11, as in cortisone-21-succinate, does not decrease inhibitory activity as much as does a C_{11}-β-OH group, such as is present in hydrocortisone-21-succinate. Alteration in the A ring, either by formation of a derivative at C-3 [testosterone-3-O-(carboxymethyl)oxime] or by aromatization of the ring (estrone and estradiol), greatly decreases or abolishes inhibitory activity. It would appear, from these results, that the structural features of ring A, that portion of the hapten molecule which is furthest removed from the point of attachment to the BSA, determines, to a great extent, the specificity of the reaction between T-17-BSA and anti-T-17-BSA. It is expected that continuation of studies of this type, by use of additional soluble steroid derivatives, will reveal more precisely the chemical basis of specificity.

An interesting example of specificity is depicted in Fig. 1, in which the inhibiting effectiveness of T-17-succinate and C-21-succinate on the reactions of C-21-BSA and T-17-BSA with an antiserum to C-21-BSA are plotted. C-21-succinate, the homologous hapten, equally inhibits the reaction with the homologous antigen, C-21-BSA, and the heterologous antigen, T-17-BSA. On the other hand, the heterologous hapten, T-17-succinate, inhibits the cross-reaction with T-17-BSA much better than it inhibits the reaction with the homologous antigen, C-21-BSA. Thus, although anti-C-21-BSA cross-reacts with T-17-BSA, by addition of a suitable concentration of T-17-succinate it should be possible to inhibit practically completely the cross-reaction with negligible effect on the homologous reaction. It is hoped that, in this manner, the in vivo activity of anti-C-21-BSA may be studied without complicating cross-reactions, and such investigations are now in progress.

It has thus been demonstrated that several steroid hormones may act as haptens when coupled to a protein, and antibodies with steroid specificity may be elicited (*5*).

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17 September 1958

Vanguard Measurements Give Pear-Shaped Component of Earth's Figure

The determination of the orbit of the Vanguard satellite, 1958β_2, has revealed the existence of periodic variations in the eccentricity of that satellite (*1*). Our calculations indicate that the periodic changes in eccentricity can be explained by the presence of a third zonal harmonic in the earth's gravitational field. The third zonal harmonic modifies the geoid toward the shape of a pear. In the present case, the stem of the pear is up—that is, at the North Pole. According to our analysis, the amplitude of the third zonal harmonic is 0.0047 cm/sec² in the surface acceleration of gravity, or 15 meters of undulation in the geoid.

Figure 1 shows the observed variation in eccentricity. The period of the variation in eccentricity is 80 days, approximately equal to the period of revolution of the lines of apsides. The eccentricity is a maximum when the perigee is in the Northern Hemisphere. The amplitude of the variation is 0.00042 ± 0.00003. Similar perturbations may exist in the angle of inclination of the orbit, although the data for them are much less accurate. No perturbations of this magnitude appear to exist in the semimajor axis.

In principle, the perturbation might be caused by both odd and even harmonics. However, the even harmonics can be excluded because the observed effect has opposite signs in the Northern and Southern hemispheres. Furthermore, we can also exclude tesseral harmonics (those which depend on longitude as well as latitude) because these also are the same in the Northern and Southern hemispheres, apart from a shift in longitude. We are left with the zonal harmonics (those which depend only on latitude) of odd degree.

Of the odd zonal harmonics, the first degree is forbidden; and those of higher degree are unlikely to have a large effect because they die out inversely as the $(n+1)$ power of the distance. The effect is therefore due mostly to the third zonal harmonic, with a possible contribution from the fifth.

Accordingly, a calculation was made of the effect of the third zonal harmonic on the orbit elements of 1958β_2, by methods developed by O'Keefe and Batchlor (*2*). In the resultant expression for the eccentricity, the dominant terms were those whose argument was the mean motion of perigee. These were larger than the others by a factor of 10^3. Keeping only the large terms, we find

$$e = e_0 + \frac{3}{2} A_{3,0} \frac{(1-e^2)^{1/2}}{na^6} \frac{1}{n'} \times \sin i \left(1 - \frac{5}{4}\sin^2 i\right) \sin \omega \qquad (1)$$

where $A_{3,0}$ represents the coefficient of the third zonal harmonic in the notation of Jeffreys (*3*), n is the orbital mean motion and n' is the mean motion of the perigee, e is the eccentricity and e_0 the mean eccentricity, i is the angle of inclination, ω is the argument of perigee, and a is the semimajor axis.

Setting in the constants of the orbit and the observed amplitude of e, we find

$$A_{3,0} = (2.5 \pm 0.2) \times 10^{29} \qquad (2a)$$

in meter-second units. Utilizing the relation given by Jeffreys,

$$A_{n,s} = \frac{c^{n+2}}{n-1}\, g_{n,s}$$

(where $A_{n,s}$ is the coefficient of the disturbing potential, $g_{n,s}$ is the acceleration of gravity at the surface of the earth, and c is the earth's equatorial radius), we find that the third zonal harmonic of gravity at the earth's surface, in milligals, is

$$g_{3,0} = 4.7 \pm 0.4 \qquad (2b)$$

Equation 2 is relevant to what Vening Meinesz (*4*) and Heiskanen call the "basic hypothesis of geodesy." These authors assume that the earth's gravitational field is very nearly that of a fluid in equilibrium. They consider that the deviations from such an ellipsoid, in any given area, do not exceed about 30 milligal-megameter units—that is, they assume that one will not find deviations of more than 30 milligals over an area of 1000 kilometers on a side, or deviations of more than 3 milligals in an area 3000 kilometers on a side.

Our determination of the third-degree zonal harmonic shows that the hypothesis of Vening Meinesz and Heiskanen is

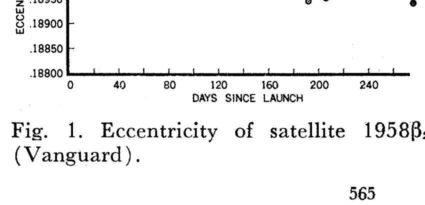

Fig. 1. Eccentricity of satellite 1958β_2 (Vanguard).