STUDIES ON THE USE OF METALS IN SURGERY

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PART I

COMPARATIVE DETERMINATIONS OF THE CYTOTOXICITY OF CERTAIN METALS IN FIBROBLAST CULTURE*

AN IDEAL METAL for use in bone surgery should be noncytotoxic, light, and so strong that the bulk of the appliance could be kept small. Its value would be still further enhanced if the physical properties were such that it not only cast well but existed in a malleable form, particularly if, in the latter state, it could be quickly hardened by heating.

A number of noncytotoxic metals and alloys are in existence, several of which are quite satisfactory for internal splinting of bone. Of these, stainless steel and Vitallium are perhaps two of the best examples. The latter has been carefully studied in both animals and man by Venable and his coworkers,[1, 2] who have convincingly demonstrated its usefulness. Our observations upon the effect of Vitallium upon the rate of growth of fibroblasts in tissue culture (see below), and, thus far, of its tolerance (in the form of plates) *in vivo,* afford further corroboration of its applicability in bone surgery.

If, at operation, the available appliance cannot be fitted satisfactorily, it would be very convenient to be able to alter its shape as desired, then quickly to harden it. This, so far as we have been able to learn, cannot be carried out with Vitallium, for it is not malleable.

While searching for a metal which would conform to the above-mentioned requirements, and which would be suitable for the repair of extensive cranial defects, our attention was called to a relatively new alloy, Ticonium. It is made up of nickel, cobalt, chromium, and molybdenum, with a small amount of beryllium added if it is to be cast. The physical properties† are quite

* A study of the use of various metals for the repair of cranial defects was undertaken at the same time, which will be published shortly.

† The metallurgic properties‡ of this alloy in its two different forms are:

(1) *Casting Alloy.*—This alloy, which is used in casting, has the following composition: Nickel 35.6 per cent, cobalt 29.1 per cent, chromium 27.7 per cent, molybdenum 6.0 per cent, beryllium 1.6 per cent. This metal has the lowest melting point of any alloy in the so-called chromium alloy series and, due to the presence of beryllium, can be cast to a high degree of fidelity. Its physical properties are as follows:

satisfactory—it is strong, rather light, and can be very accurately cast or wrought.

We have been studying the tolerance of Ticonium, by living tissues, and hence its applicability in surgery, both *in vitro* and *in vivo*. The present communication is an account of our observations of the effect of Ticonium, Vitallium, and other metals upon the growth of fibroblasts in tissue cultures. A subsequent report will record our studies of local tissue response to plates and screws in the dog's skull. The evidence thus far obtained, indicates that Ticonium, like Vitallium, silver, gold, and stainless steel, is not cytotoxic.

Method.—Ménégaux, Odiette, and coworkers[3, 4, 5, 6, 7, 8] have conclusively shown that the cytotoxicity of metals can best be studied in tissue cultures. Lately, the method of these authors has also been used by G. Krull,[9] and by A. H. Smook and Gaillard.[10]

The determination of the cytotoxicity of the metals, as carried out in the present study, is based upon the rate of growth of pure fibroblast cultures (obtained from the heart of a chicken embryo) in the presence of different metals. All these observations have been made on different generations of one and the same fibroblast strain. This strain was started February 21, 1940, and was discontinued June 21, 1940, in its forty-first generation. In order to assure growth of pure fibroblasts for actual determinations, we did not use any generations previous to the tenth generation.

Metal disks§ were prepared similar to those described by Ménégaux, Odiette, and Moÿse.[3] Each disk measured 1.5 Mm. in diameter, and 0.25

Rockwell hardness C 30; yield point 65,000–68,000 lbs. per sq. in.; ultimate strength 90,000–93,000 lbs. per sq. in.; elongation 6 per cent. The alloy may be heat treated if desirable to be hardened or softened. During the hardening treatment, the alloy is heated to 2,000° F., quenched, and then drawn to 1,300° F. The alloy then increases in hardness to Rockwell C 40–45 and the yield point also raises to 85,000–95,000 lbs. per sq. in. This alloy in the hardened condition is quite brittle. During the softening treatment, the alloy is drawn to 2,000° F. The alloy then softens to Rockwell C 18 and the yield point drops to about 45,000 lbs. per sq. in. In the softened condition, the alloy can easily be worked or machined if desired.

(2) *Wrought Alloy.*—The alloy which is used in wrought stock has the following composition: Nickel 36.2 per cent, cobalt 29.6 per cent, chromium 28.2 per cent, molybdenum 6.0 per cent. Its properties depend upon the amount of cold work done after the last anneal. For example, stock drawn to 50–60 per cent cold reduction in area after final anneal: Ultimate strength 237,000 lbs. per sq. in.; yield point 177,000 lbs. per sq. in.; elongation 6 per cent. Stock drawn to a 30 per cent cold reduction in area after final anneal: Ultimate strength 220,000 lbs. per sq. in.; yield point 183,000 lbs. per sq. in.; elongation 6 per cent. Stock drawn and fully annealed: Ultimate strength 150,000 lbs. per sq. in.; yield point 96,000 lbs. per sq. in.; elongation 31 inches on ten inches gauge length (52 per cent on two inches gauge length); hardness Rockwell B 95.

‡ The metallurgic data were made available by Mr. E. Touceda from the Touceda Laboratories, Albany, N. Y.

§ The metal disks were kindly prepared by the Touceda Laboratories in Albany, N. Y., and the Vitallium by the Austenal Laboratories in New York City.

Mm. in thickness. We have carried out our determinations on eight different metals, namely, gold, silver, Ticonium (casting alloy), Ticonium (wrought alloy), Vitallium, stainless steel, vanadium and copper.

Throughout the various experiments, we have compared the different metals with each other as well as with control cultures containing no metal. As an additional control, we have used cultures containing gold disks, as gold has proved to be nontoxic to fibroblast cultures (Ménégaux and associates[7]).

Technic.—Explants from the heart muscle of an 11-day-old chick embryo were prepared and cultivated by means of the hanging-drop technic. The medium used consisted of chicken plasma, chick embryo extract and Tyrode solution. The blood necessary for the preparation of the plasma was obtained under sterile precautions from young roosters by cardiac puncture. Heparin (1 mg. per 1 cc. saline) was used in order to prevent coagulation. The blood was then centrifuged for ten minutes at 2,300 R.P.M. After centrifuging, the supernatant plasma was withdrawn with pipettes. Small amounts of plasma were placed in previously autoclaved test tubes and stored in the refrigerator. Fresh plasma was prepared every two to three weeks.

For the preparation of embryonic tissue extract, eggs were incubated for eight or nine days at a temperature of 38.5° C. Twenty-four hours before the transplantation, the embryo was removed to a sterile watch-glass and reduced to a pulp by the use of a fine scissors. The undiluted pulp was then stored in the refrigerator. Immediately before the transplantation, the extract was centrifuged for ten minutes at 2,300 R.P.M. The supernatant fluid was withdrawn and mixed with Tyrode solution (3:0 dilution).

The Tyrode solution was prepared as described by Parker.[11] The *p*H of the solution was kept at 7.4–7.6. We have found that it is of the utmost importance to keep the *p*H at this level, for the rate of growth, as well as the type of growth, was influenced immediately whenever the *p*H was changed. The *p*H was checked colorimetrically at frequent intervals. The completed mixture was passed through a Seitz filter and was then stored in small amounts in the refrigerator in rubber-stoppered test tubes. For transplantation, each culture was divided into two or four approximately equal parts, and each part placed in a drop of plasma upon a new coverslip. One equal-sized drop of embryonic tissue extract, which had been mixed previously with Tyrode solution (3:1), was added and the hanging-drop culture sealed upon a round depression slide by the use of vaseline. As soon as coagulation had taken place, the cultures were placed in an incubator at a temperature of 38.5° C. Every two or three days these cultures were transferrd to a new medium until finally nothing but fibroblasts were growing. With the tenth generation we started our experiments. Each transplant of this generation was divided into four equal pieces. These pieces of tissue were implanted in a hanging-drop. A Ticonium disk was added to the first transplant, a gold disk to the second, a copper disk to the third, and the fourth transplant was cultivated without any metal. The disks were placed fairly close to the tissue (Figs.

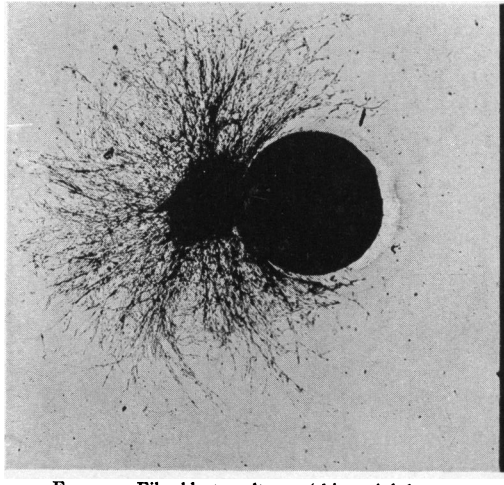

FIG. 1.—Fibroblast culture (thirty-eighth generation) in presence of a silver disk. (Hansen's hematoxylin stain. [×23])

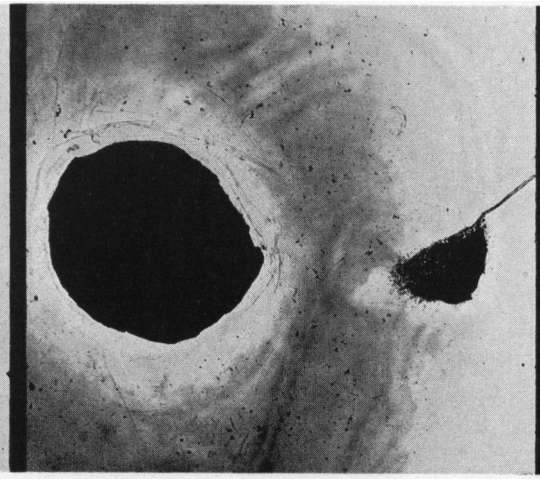

FIG. 2.—Fibroblast culture (eleventh generation) in presence of a copper disk. No growth. Note distance between transplant and copper disk. (Hansen's hematoxylin stain. [×34])

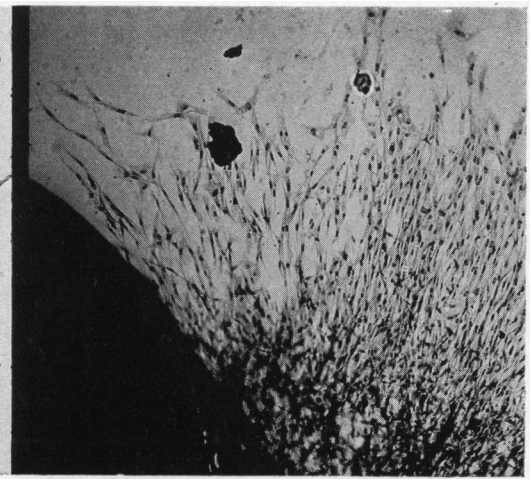

FIG. 3.—Section of fibroblast culture (eleventh generation) in presence of a Ticonium disk. A part of the disk is visible in the left lower corner. Note the growth of cells along the edge of the metal. (Hansen's hematoxylin stain. [×112])

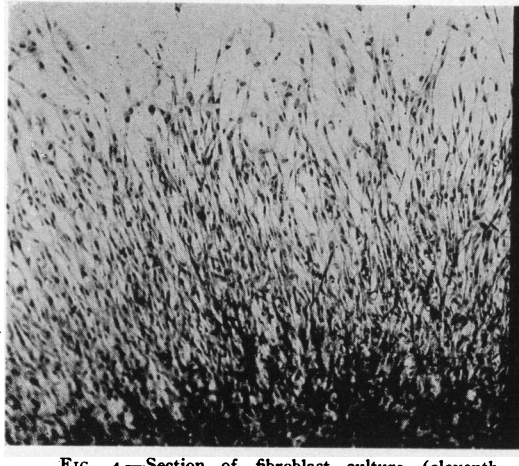

FIG. 4.—Section of fibroblast culture (eleventh generation) in presence of Ticonium disk. (Hansen's hematoxylin stain. [×112])

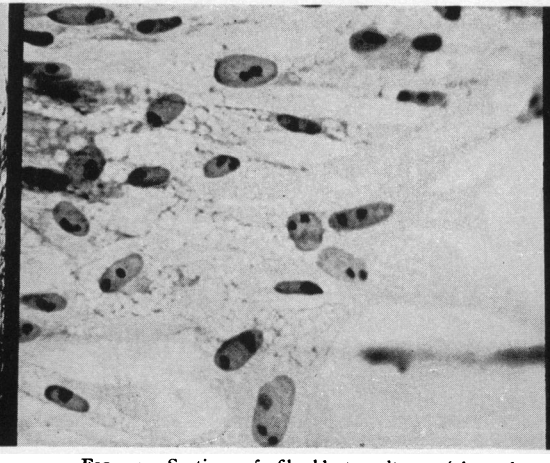

FIG. 5.—Section of fibroblast culture (eleventh generation) in presence of Ticonium disk. (Hansen's hematoxylin stain. [×880])

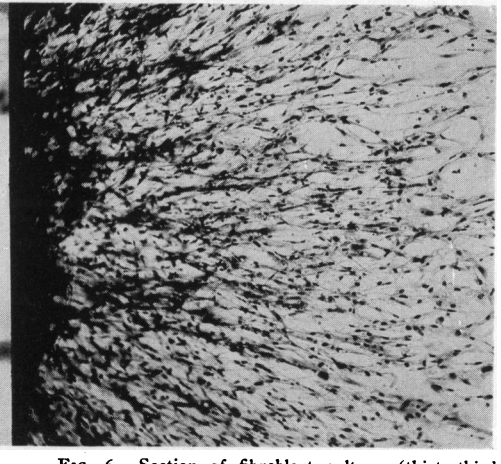

FIG. 6.—Section of fibroblast culture (thirty,third generation) in presence of Vitallium disk. (Hansen's hematoxylin stain. [×112])

1 and 3). After coagulation had taken place, the outline of each culture was drawn by use of the camera lucida and placed in the incubator immediately afterward. After exactly 48 hours, the cultures were again drawn by use of the camera lucida at the same magnification. Subsequent experiments were carried out in a similar manner.

By use of a planimeter, the area of the original fragment as well as the total area was measured. The absolute increase of the fragment was obtained by subtracting the area of the original fragment from the total area. The absolute increase of the fragment was then divided by the area of the primitive fragment, thus determining the relative increase of the fragment (Ebeling[12]). This value we have called the "factor of growth."

We realize that this method of surface measurements fails to take into consideration the growth in the third dimension; however, we have felt it to be of value to show these figures for they are a means of expressing all the significant variations which we have seen in this comparative study of cytotoxicity.

TABLE I

ELEVENTH GENERATION

	Relative Increase of the Fragment in the Presence of:			
	Ticonium (casting alloy)	Gold	Copper	Control
	11.00	11.00	0.00	14.90
	8.87	4.16	....	12.00
	5.81	9.10	....	7.97
	14.80	7.80	0.00	13.10
	.....	6.00	0.00	9.90
	12.40	9.40	0.00	11.70
	13.40	11.60	....	14.00
	.....	10.70	....	9.00
	4.20	7.40	....	6.30
Averaged Factor	**10.07**	**8.57**	**0.00**	**10.98**
Maximum	*+4.73*	*+3.03*	....	*+3.92*
Minimum	*−5.87*	*−4.41*	....	*−4.68*

TABLE II

THIRTY-THIRD GENERATION

	Relative Increase of the Fragment in Presence of:			
	Ticonium (wrought alloy)	Vitallium	Vanadium	Control
	7.8	5.1	0.00	5.3
	8.2	6.6	0.00	6.8
	7.4	7.0	0.00	6.8
	10.1	9.1	....	...
	10.6	...	....	...
	15.1	...	....	...
Averaged Factor	**9.9**	**7.0**	**0.00**	**6.3**
Maximum	*+5.2*	*+2.1*		*+0.5*
Minimum	*−2.5*	*−1.9*		*−1.0*

Experiments.—In every experiment, the average factor of growth of the fibroblast cultures for each metal was calculated. Table I shows the growth factors of the fibroblast cultures in their eleventh generation; Table II, those of the cultures in their thirty-third generation.

Of the 41 generations, 13 generations were chosen for our determina-

tions. A total of 222 fibroblast cultures have been examined; 31 control cultures without any metal disks have been examined and measured; gold was tested in 24 cultures; silver in 21; Ticonium (casting alloy) in 40; Ticonium (wrought alloy) in 31; Vitallium in 13; stainless steel in 23; vanadium in 21 and copper in 18 cultures.

The average factor of growth of the fibroblast cultures for each metal, as obtained from the different generations, used thus for study, is summarized in Table III.

TABLE III

AVERAGE OF THE RELATIVE INCREASE OF THE FRAGMENT ("FACTOR OF GROWTH")

Generation	No. of Cultures	Ticonium (cast)	Ticonium (wrought)	Gold	Silver	Vitallium	Stainless Steel	Vanadium	Copper	Control
11, a	29	10.07	..	8.57	..	..	..	..	0.00	10.98
11, b	13	8.60	..	8.50	..	..	..	..	..	..
15	14	10.80	..	9.40	..	..	..	..	..	..
18	4	..	..	..	..	..	..	0.00	..	7.74
19	16	..	5.70	..	..	..	..	..	..	5.70
25	30	6.00	..	..	..	..	8.93	1.80	..	..
26	16	..	6.40	9.20	..	..	6.34	..	..	..
28	13	..	24.10	..	..	..	8.90	0.00	0.00	..
29	15	7.50	8.90	..	..	..	..	..	0.00	..
33	16	..	9.90	..	..	7.00	..	0.00	..	6.30
38	21	..	..	..	8.90	..	..	..	..	8.00
39	19	..	7.05	..	8.94	..	5.28	1.77	..	..
41	15	..	23.18	..	13.00	16.49	..	..	..	..
Averaged Factor		**8.59**	**9.31**	**8.91**	**10.28**	**11.74**	**7.36**	**0.71**	**0.00**	**7.74**
Maximum		*+2.21*	*+14.79*	*+0.49*	*+2.72*	*+4.45*	*+1.57*	*+1.09*		*+3.24*
Minimum		*−2.59*	*− 3.61*	*−0.41*	*−1.38*	*−4.74*	*−2.08*	*−0.71*		*−2.04*

A number of cultures were fixed in neutral Ringer-formol solution (Parker[11]), and have been stained with Hansen's hematoxylin (Figs. 1, 2, 3, 4, 5, 6) for purpose of illustration.

Before we started the strain used for the evaluations given above, we studied the influence of different metals upon the rate of growth of about 200 fibroblast cultures, which were discarded after observation. The results of these single experiments correspond with our findings obtained from the strain described above.

(1 *Control Cultures.*—For five different generations we prepared a total of 31 cultures, cultivated without any metal disk in the medium. Table III shows the average factors of growth for the different generations. The average factor of growth for all 31 cultures was calculated to be 7.74. It is well-correlated with the factors obtained for those metals that we will show to be nontoxic. The difference between these control cultures and the two toxic metals (vanadium and copper) in our series is obvious.

(2) *Gold and Silver.*—As stated above, for five different generations, we prepared a total of 31 cultures without any metal disk. In the remaining eight generations, we have used metals as controls, for instance, gold which has been proved to be noncytotoxic (Ménégaux and Odiette[7]). We thought that it would be interesting to compare the growth in the presence of these

nontoxic metals with the growth in the presence of those metals which we have been investigating for their possible use in surgery.

For four generations we cultivated fibroblasts in the presence of gold disks. This represented a total of 24 cultures. The average of the relative increase of the fragment amounted to 8.91. Silver disks were implanted in 21 cultures belonging to three different generations. The average factor of growth amounted to 10.28.

(3) *Ticonium.*—For five generations we prepared cultures with the "casting alloy" Ticonium, representing a total of 40 fibroblast cultures. The factor of growth averaged 8.59. The factor of growth for the "wrought alloy" Ticonium was 9.31. It was obtained from a total of 31 cultures studied in seven different generations.

(4) *Vitallium.*—Thirteen cultures were used for testing the alloy vitallium. These 13 cultures were chosen from only two different generations. In the thirty-third generation the average factor of 7.00 was obtained (Table II). For the forty-first generation, the factor for Vitallium was 16.49. In the forty-first generation, however, the factor for silver was 13 while that of the "wrought alloy" Ticonium was 23.18.

The fibroblast cultures for this generation obviously grew luxuriantly, thus raising the average factor of growth above the level obtained in other experiments. That the average area of growth for Vitallium amounted to 11.74, may be explained by the fact that Vitallium was tested in this generation and in one other only, whereas the "wrought alloy" Ticonium was tested in seven different generations.

(5) *Stainless Steel.*—This metal was tested in a total of 23 cultures obtained from four different generations. The average factor was 7.36.

(6) *Vanadium.*—Twenty-one cultures were tested with Vanadium in five different generations. Thirteen of these cultures did not grow at all. The remaining eight showed a marked inhibition of growth, the average factor of growth was 0.71. Clinically, the failure of this metal has been reported repeatedly. We have now obtained proof *in vitro* for the high cytotoxity of this alloy.

(7) *Copper.*—Copper was tested in three different generations with a total of 18 cultures. None of these cultures showed any growth of fibroblasts.

Discussion.—The evaluation given above draws a definite line between the six nontoxic metals and vanadium and copper, both of which prove to be highly cytotoxic. We have based our determinations on one criterion—the actual growth of fibroblasts in the presence of metals.

Copper in its pure form may be considered completely cytotoxic, since no growth was obtained in any of the cultures containing it. Vanadium may be regarded somewhat less cytotoxic than copper, for a slight growth was obtained in eight of 21 cultures and no growth at all in the remaining 13. The cytotoxicity of vanadium in our cultures is of such a degree as to make the

use of this substance in bone surgery inadvisable. Its cytotoxicity is the probable explanation of the failures reported of its use in clinical surgery.

Five of these substances (gold, silver, Vitallium, and two Ticonium compounds) gave a factor of growth higher than the control cultures without metal disks. Stainless steel gave a result slightly higher than the controls without disks.

We do not feel that any conclusive deductions can be drawn from the varying rates of growth of the metals we have found to be noncytotoxic.

SUMMARY

(1) The cytotoxicity of the eight metals (gold, silver, Ticonium (casting alloy), Ticonium (wrought alloy), Vitallium, stainless steel, vanadium, and copper) was determined by measuring the area of growth of fibroblast cultures in the presence of these metals.

(2) Gold, silver, Ticonium (casting alloy), Ticonium (wrought alloy), Vitallium and stainless steel are found to be nontoxic. Vanadium and copper are found to be highly cytotoxic.

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