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44. Hisashi Nogami and Manabu Hanano : Studies on Percutaneous Absorption. II. Effect of the Incorporated Substance.†

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The effect of both the substance which is absorbed through the skin and the vehicle used with it has been discussed in many previous investigations of percutaneous absorption through the intact skin. According to the lipid theory of Meyer and Overton[1] on the percutaneous absorption, the mechanism of superficial barrier had been made clear in a physiological investigation by Starkenstein.[2] The lipid theory postulates that the cell membrane consists of a lipid, because only a lipid-soluble substance may penetrate into the cell and penetration of drugs depends on the degree of solubility in the lipid.

On the contrary, the influence of an incorporated substance is usually very small and there is no rule to it as was pointed out by many previous experiments. Rothman[3] said in his book that the kind of vehicle in which a drug is incorporated and its solubility in the vehicle are of secondary importance. Some of the remarkable effects of vehicles and solvents on absorption are first, organic solvents promote the penetration of drugs, second, the wetting agents increase the absorption, and the vehicle in which polyethylene glycol is incorporated decreases the penetration of drugs.

It may be considered that an organic solvent has a promoting effect when the penetrating substance dissolves in lipid, but when lipid-insoluble substances are absorbed through the intact skin, it may be necessary to break the barrier of the skin for absorption of drugs. Luduena, *et al.*[4] found a pronounced promoting effect of a solvent mixture consisting of diethyleneglycol monoethyl ether and octyl alcohol on the absorption of mecholyl and epinephrine salts, but not for other drugs. Valette and Cavier[5] found that water-soluble salts, as well as lipid-soluble substances, are better absorbed if dissolved in eucalyptol than from alcoholic solutions. Rothman and Flesch[6] said, however, that with a substance to which the skin is completely impermeable, one will be unable to enforce absorption with any kind of ointment. Miescher[7] noted that absorption of lipid-insoluble materials is not promoted by organic solvents.

Surface active agents decrease the surface tension of a liquid and the capillary penetration is promoted, because the degree of wetting is enforced. It is very doubtful whether the percutaneous absorption of drugs is promoted by the same mechanism. There are, however, some investigations in which it is found that many of the washable base containing wetting agents may have a considerable effect on promoting absorption. Duemling[8] and Laug, *et al.*[9] found that some emulsion bases containing wetting agents promote the absorption of drugs, but even the wetting agent does not always give

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† The title of previous report "Studies on Ointments. I "(This Bulletin, **4**, 347(1956)) is changed to "Studies on Percutaneous Absorption. I."

1) G. Fischer : "Studien über die Narkose," Jena (1924).
2) E. Starkenstein, F. Hendrych : Arch. exptl. Pathol. Pharmakol., **182**, 664(1936).
3) S. Rothman : "Physiology and Biochemistry of the Skin," University of Chicago Press (1954).
4) E. Luduena, J. Fellows, R. Driver : Arch. Dermatol. u. Syphilis, **57**, 210(1948).
5) G. Valette, R. Cavier : Compt. rend. soc. biol., **139**, 904(1945).
6) S. Rothman, P. Flesch : Ann. Rev. Physiol., **6**, 195(1944).
7) G. Miescher : Dermatologica, **83**, 50(1941).
8) W. Duemling : Arch. Dermatol. u. Syphilis, **43**, 264(1941).
9) E. Laug, E. Vos, F. Kunze : J. Pharmacol. Expttl. Therap., **89**, 52(1947).

a demonstrable effect. None was found by Strakosch and Clark[10] for sulfonamides, and none by Shelly and Melton[11] for histamine base and histamine salts. Zopf, *et al.*[12] found that wetting agents have no promoting action in the absorption of potassium iodide and of phenolsulfophthalein from various bases by the intact skin of albino rats.

Percutaneous absorption usualy diminishes on using vehicles containing polyethylene glycol. Kobori, Miyazaki, and others[13] found that salicylic acid and diphenhydramine base are lipid-soluble and of course permeable through the intact skin, but they become impermeable when polyethylene glycol ointment is used. So they concluded that polyethylene glycol ointment is an impermeable vehicle. This action of polyethylene glycol is very interesting, because the effect of vehicles and solvents demonstrated in the previous investigations has a little influence on the absorption of drugs.

A drug must be soluble in the lipid for penetration through intact skin by the lipid theory, so that the obstructing effect of polyethylene glycol on the percutaneous absorption may be explained from physical chemistry. Of course, partition between the vehicle and lipid of the skin may be considered as the replacement of drugs from polyethylene glycol to the lipid. Salicylic acid dissolves perfectly in polyethylene glycol and then the reduction of partition may be presumed when the decrease of absorption is recognized. If the apparent partition coefficient between vehicles and suitable solvents is determined, the partition may be parallel with the percutaneous absorption of this acid. It was found after the test of several solvents that benzene was the most suitable solvent for this purpose. The purpose of this investigation is to discuss the absorption of drugs through the intact skin in relation to some physicochemical constants.

In this experiment the absorption of salicylic acid through the intact human skin was examined incorporating it with distilled water, and aqueous solutions of polyethylene glycol 400, Carbowax 6000, propylene glycol, glycerol, and glucose in various concentrations. On the other hand, the apparent partition of salicylic acid between benzene and these solutions, their viscosities, and their surface tension were measured. From the results obtained, it was proved that the apparent partition between the vehicle and the lipid is a very important factor in percutaneous absorption.

Experimental

Material—Salicylic acid was added to each of the following 22 vehicles to make 0.2(w/w)% solution and kept in containers. The vehicles were 10, 20, 30, 40, and 50(w/w)% aqueous solutions of polyethylene glycol 400, and 10, 20, 30, and 40(w/w)% aqueous solutions of Carbowax 6000, propylene glycol, glycerol, and glucose.

Design—Internal side of both forearms of adult male were used for the measurement of the absorption of drugs. The residual measuring method was chosen in this study to measure absorption, because this method had been proved to be more precise than the urinary excretion method as shown in the preceding report.† According to the result of a preliminary experiment, the amount of absorption increased suddenly when drugs were applied repeatedly to the same place. For the reason of this change it may be concluded that the skin of that portion had been injured by stimulation of drugs and it required at least one week to recover. The variance is so great that an experimental design to reduce them as much as possible is desirable for the investigation of percutaneous absorption, in spite of a better precision of the residual measuring method. This experiment on the absorption was divided into two parts. One was for the following six kinds of vehicles. Distilled water and 10, 20, 30, 40, and 50(w/w)% aqueous solutions of polyethylene glycol. This was designed using randomized block design. Each vehicle is applied once to each person, each of all substances being tested five times. Two different drugs were applied to one arm at the same time and then a repeat of application had to be made on the same arm to finish the experiment of

10) E. Strakosch, W. Clark : Am. J. Med. Sci., **206**, 610(1943).
11) W. Shelley, F. Melton : J. Invest. Dermatol., **13**, 61(1949).
12) D. Meyer, M. Nadkarni, L. Zopf : J. Am. Pharm. Assoc., Sci. Ed., **38**, 23(1949).
13) T. Kobori, J. Miyazaki : Yakuzaibuchokai-Nempo, **11**, 177(1952).

six substances. Therefore, an interval of more than a week was allowed for the next application.

Another part was for the rest of 16 kinds of vehicles. If this experiment were designed the same way as above, it will require a long time, for the recovery of the tissue takes a very long time. The experiment was designed by a kind of lattice design to shorten the term for perfection. These 16 kinds of solution were arranged into 4×4 square lattice and then four solutions, which were held in each of four columns, were applied to each of four men. In this experiment there were five lattices and so 20 men were used. Each solution was tested five times and variances between men were excepted as well as the randomized block design. Moreover, four solutions were applied at the same time once to each man and so it was not necessary to apply each preparation to the same portion repeatedly.

Measurement of Percutaneous Absorption—One g. of each solution was placed in a polyethylene cup (almost full) of 1.5 cm. diameter and the cup was inverted on the forearm of a healthy adult male and fastened with adhesive plaster. After 16 hrs. the solution, the adhesive plaster, and the cup were removed and the solution remaining on the skin was repeatedly washed off. These were extracted completely with warm 1% NaOH solution and made up to 50 cc. with distilled water. To 25 cc. of the solution in a glass-stoppered test tube, 1 cc. of 36% HCl and 9 cc. of $ClCH_2CH_2Cl$ were added, shaken vigorously, and centrifuged. Aqueous layer was taken off, 6 cc. of $ClCH_2CH_2Cl$ was transferred to another test tube, 10 cc. of distilled water and 0.5 cc. of iron test reagent (1% iron alum in 0.05*N* HCl) were added, shaken, and centrifuged. Upper aqueous layer was transferred to a cell and its optical density was read, using an electrophotometer with S-51 filter. Amount of percutaneous absorption is determined by subtracting this result from 2 mg. of salicylic acid.

Measurement of Apparent Partition Coefficient of Salicylic Acid between Benzene and Aqueous Solution—A mixture of 5 cc. of solution and the same volume of benzene placed in a glass-stoppered test tube was shaken in a water bath at 37° for 6 hrs. and then kept vertically in the bath over night. To 2 cc. of the benzene layer, 10 cc. of water and 1 cc. of 0.1*N* NaOH were added, shaken, and centrifuged. The benzene was discarded, 5 cc. of the aqueous layer was transferred to another tube, 1 cc. of conc. HCl and 9 cc. of $ClCH_2CH_2Cl$ were added, shaken, and centrifuged. To 6 cc. of the $ClCH_2CH_2Cl$ layer 10 cc. of water and 0.5 cc. of iron test reagent were added, shaken, and centrifuged. The supernatant, colored aqueous layer was transferred to a cell and its optical density was measured.

To 2 cc. of the initial aqueous layer, 8 cc. of water and 1 cc. of 0.1*N* NaOH were added, shaken, and optical density of 5 cc. of this solution was measured in the same way as the benzene layer. The apparent partition coefficient of salicylic acid was calculated by the division of both concentrations.

Measurement of Viscosity and Surface Tension of the Solution—Viscosity was measured by the Ostwald and Stokes apparatus at 37°. Surface tension was measured by the Du Noüy apparatus at room temperature.

Results and Discussion

Original data of percutaneous absorption are shown in Tables I and II, and apparent partition coefficient, viscosity, and surface tension are listed in Table III.

Fig. 1a gives the relationship between the kind of solution and its concentration in absorption. Tables IV and V give the analyses of variance on data of Tables I and II, respectively.

As seen in Fig. 1a, and Tables IV and V, percutaneous absorption is different from each solution. Polyethylene glycol 400 and Carbowax 6000 show the largest obstructive effect on percutaneous absorption. Propylene glycol and glycerol also show the same effect, and that of propylene glycol is larger than that of glycerol at statistically significant level but that effect is not found in glucose solution. Each solution which shows the obstructive effect increases the effect with concentration. As seen in these Tables of analysis of variance, the variance which depends upon the individual is statistically significant. The confidence limit of each mean value of absorption in the first experiment was 22.4 and that of each corrected one in the second was 76.2 (unit γ). The differences between the simple mean values of absorption and these corrected with variance depended on the individual were so many that the precision of experiment increased by about 50% as compared with that disregarding the variance. It is

Table I. Absorption from Polyethylene Glycol 400 (unit, 10 γ)

Man. \ PEG%	0	10	20	30	40	50
1	112	82	62	40	22	22
2	114	90	61	41	18	10
3	132	106	80	56	44	16
4	94	72	64	36	28	12
5	96	80	82	48	30	20
No. of Tr.	0	1	2	3	4	5

Table II. Absorption from Carbowax 6000, Propylene Glycol, Glycerol, and Glucose Solution

(Unit, 10 γ. No. of treatment in parentheses)

Concn.	No. of Treatment 10%	20%	30%	40%
Carbowax	11	12	13	14
Propylene glycol	21	22	23	24
Glycerol	31	32	33	34
Glucose	41	42	43	44

Data of Absorption

Lattice 1

1	(11) 56	(32) 98	(24) 78	(41) 108
2	(43) 130	(22) 119	(21) 124	(34) 60
3	(23) 96	(33) 66	(31) 124	(42) 136
4	(12) 46	(13) 22	(14) 20	(44) 84

Lattice 2

5	(21) 146	(24) 112	(14) 46	(31) 124
6	(13) 44	(33) 82	(22) 100	(32) 110
7	(42) 92	(44) 88	(41) 108	(34) 82
8	(11) 92	(43) 120	(23) 90	(12) 58

Lattice 3

9	(23) 84	(41) 104	(14) 60	(22) 88
10	(44) 108	(43) 108	(33) 90	(24) 84
11	(21) 98	(13) 30	(11) 104	(42) 108
12	(32) 86	(31) 104	(34) 80	(12) 80

Lattice 4

13	(22) 104	(12) 32	(24) 80	(42) 112
14	(44) 134	(21) 104	(23) 92	(32) 100
15	(11) 114	(33) 92	(34) 88	(14) 50
16	(31) 120	(41) 104	(13) 44	(43) 98

Lattice 5

17	(43) 106	(14) 16	(32) 98	(42) 83
18	(21) 104	(12) 64	(41) 98	(33) 50
19	(34) 46	(13) 16	(24) 60	(23) 72
20	(22) 136	(44) 114	(11) 84	(31) 92

Table III. Partition Coefficient, Viscosity, and Surface Tension

No. of Treatment	Part. Coeff.	Viscosity	Surf. Tens.	No. of Treatment	Part. Coeff.	Viscosity	Surf. Tens.
0	1.69	1.00	791	22	1.35	1.60	764
1	1.12	1.45	813	23	1.35	2.21	750
2	0.676	2.22	807	24	1.23	3.16	752
3	0.476	3.48	791	31	1.34	1.32	770
4	0.363	5.73	787	32	1.20	1.79	780
5	0.263	11.20	783	33	0.806	2.44	742
11	1.02	4.84	761	34	0.537	3.36	697
12	0.604	16.59	777	41	1.75	1.27	769
13	0.348	46.86	806	42	1.86	1.75	818
14	0.192	118.43	795	43	1.99	2.70	708
21	1.41	1.09	761	44	2.00	4.87	813

Partition coefficient = concn. in benzene/concn. in solution
Viscosity = Relative viscosity with water (35 c.) as 1.00
Surface tension = Relative surface tension with water (35 c.) as 1000.

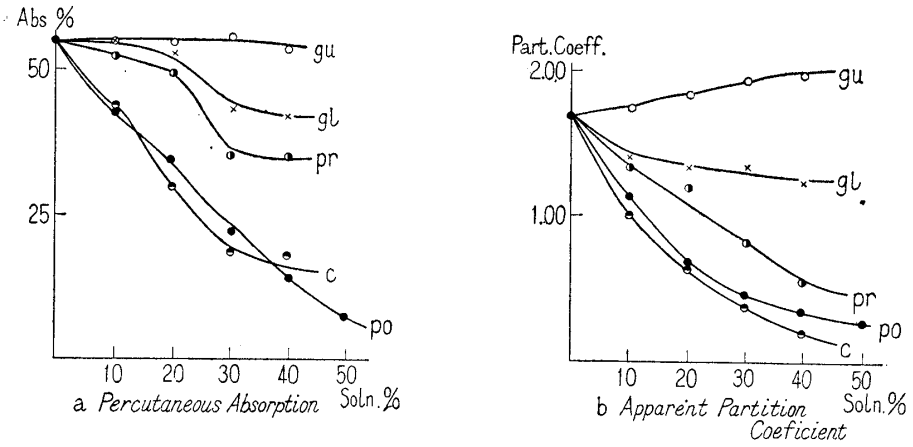

Fig. 1. Relation between Absorption and Concentration, and Apparent Partition Coefficient (a) and Concentration (b)
po : Polyethlene glycol c : Carbowax gl : Glycerol
pr : Propylene glycol gu : Glucose

TABLE IV. Analysis of Variance for Data of Table I

Factor	S.S.	D.F.	M.S.
Man	1551	4	388*
Treatment	32052	5	6410**
Error	241	20	12

* Significant at 5% level. ** Significant at 1% level.

TABLE V. Analysis of Variance for Data of Table II

Factor	S.S.	D.F.	M.S.
Lattice	2504	4	626
Treat.(uncorrected)	52875	15	3525
Man(corrected)	6348	15	432
Within man	4592	45	104
Total	241	79	

$$\mu=\frac{(432-102)}{16\times 432}=0.0480 \qquad E_e'=102\times(1+0.048)\fallingdotseq 122$$

possible to increase the precision of experiment in the case of an incomplete block design like lattice design when the absorption can be measured by the recovering method, as it is possible to apply drugs at the same time.

TABLE VI. Mean Values and Corrected Mean Values (unit γ)

No. of Treatment	Uncorrect.	Correct.	No. of Treatment	Uncorrect.	Correct.
11	900	868	31	1128	1058
12	560	596	32	984	1000
13	312	366	33	760	708
14	384	368	34	716	712
21	1152	1108	41	1044	1086
22	1098	1082	42	1062	1090
23	868	868	43	1124	1122
24	828	850	44	1056	1072

Scatter diagrams were made in order to find the relation among absorptions and each of partition coefficient, viscosity, and surface tension. These diagrams are shown in Figs. 2, 3, and 4, respectively, and Tables VII, VIII, and IX present the analysis of regression of each diagram.

As seen in these diagrams and tables the evident regression is found between absorption and partition coefficient. The percutaneous absorption decreases in straight line with the depression of partition coefficient, i.e., with decreasing concentration of

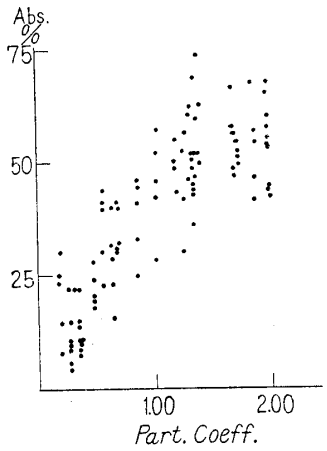

Fig. 2. Scatter Diagram Absorption-Partition Coefficient

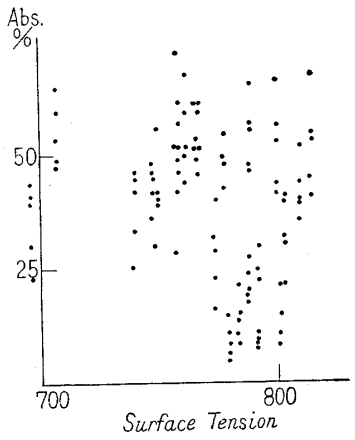

Fig. 3. Scatter Diagram Absorption-Viscosity

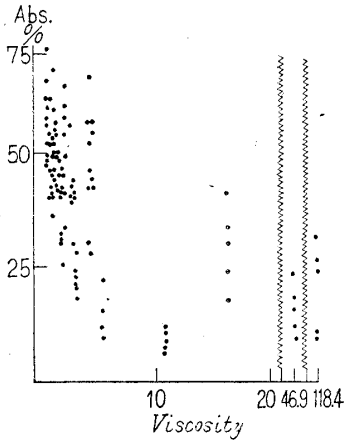

Fig. 4. Scatter Diagram of Absorption-Surface Tension

TABLE VII. Analysis of Regression for Partition-Absorption

Factor	S.S.	D.F.	M.S.
Regression	90783	1	90783**
From Regression	31941	108	296
Total	122724	109	

TABLE VIII. Analysis of Regression for Viscosity-Absorption

Factor	S.S.	D.F.	M.S.
Regression	423	1	423
From Regression	122301	108	1132
Total	122724	109	

TABLE IX. Analysis of Regression for Surface Tension-Absorption

Factor	S.S.	D.F.	M.S.
Regression	161	1	161
From Regression	122563	108	1135
Total	122724	109	

salicylic acid in benzene layer. This relation is shown more evident in Fig. 1 that presents the graph of absorption (a) and partition coefficient (b). The regression between absorption and viscosity or surface tension is not found, as will be seen in those diagrams and tables. It may be expected that osmotic pressure is the other factor related to absorption, but the effect is not found, because absorption does not change with concentration in the glucose solution.

It is impossible to measure a partition coefficient between lipid of the skin and solution of a vehicle, but it is possible to express the depression of the absorption estimated in this experiment as being attributable to the different concentration of salicylic acid between the lipid and these solutions, because absorption from the solution is parallel to partition between benzene and solution. The result reported by Kobori and Miyazaki, *et al.*, in which salicylic acid is not absorbed from polyethylene glycol ointment, indicates that the extreme depression of absorption is caused by decreased partition.

Summary

1. The percutaneous absorption of salicylic acid from several solutions was influenced by the material incorporated in it. The largest obstructive effect was shown in the case of polyethylene glycol 400 and Carbowax 6000. The same effect was shown

in propylene glycol and glycerol, and the effect of propylene glycol was larger than that of glycerol. Glucose did not show any effect. The absorption decreased with concentration of the material which showed a pronounced obstructive effect.

2. Degrees of absorption from various aqueous solutions were parallel to the degree of partition between benzene and their solution.

3. Effect of viscosity and surface tension on abosorption was not shown within the region of this experiment.

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45. **Hayao Nawa** : Studies on the Components of *Rhodea japonica* Roth. XI. Structure of Rhodeasapogenin. (4).*

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Several years ago the author isolated rhodeasapogenin, a new steroidal sapogenin, from leaves of *Rhodea japonica* Roth. and presumed its structure to be 5β,22b-spirostane-2β,3α-diol [Chart 1(A)].[1] This presumption was drawn from the following facts: (1) Rhodeasapogenin (A) isomerizes into isorhodeasapogenin (B); (2) rhodeasapogenin is readily led to derivatives of pregnenolone; (3) oxidation of rhodeasapogenin with chromium trioxide gives rhodeasapogenic acid, a dicarboxylic acid, which has a melting point similar to that of texogenic acid (D); (4) rhodeasapogenin and its derivatives are not precipitated with digitonin and do not produce acetonide; and (5) isorhodeasapogenin agrees with neither of the homologs (C) of gitogenin but has properties akin to those of *epi*-samogenin.

Later, description about texogenic acid was found incorrect and the compound

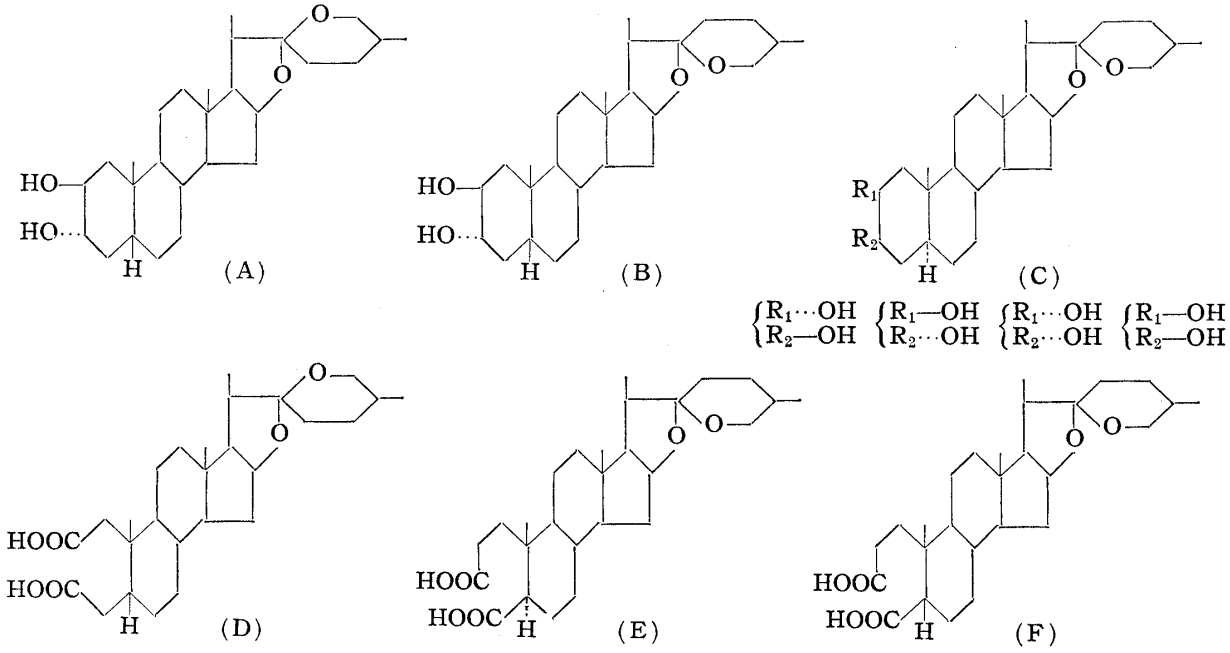

Chart 1.

* Preliminary communication, H. Nawa : Proc. Japan Acad., **33**, 570(1957).

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1) H. Nawa : Proc. Japan Acad., **29**, 214(1953); Yakugaku Zasshi, **73**, 1192, 1195, 1197(1953).