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Hematology and blood chemistry in saturation diving: I Antiplatelet drugs, aspirin, and VK744

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Philp, R. B., D. Freeman, I. Francey, and B. Bishop. 1975. Hematology and blood chemistry in saturation diving: I Antiplatelet drugs, aspirin, and VK744. Undersea Biomed. Res. 2(4):233-249.–Blood chemistry and cellular parameters were studied before, during, and after saturation (2.4 ATA) dives in the HYDRO-LAB habitat on two separate occasions. In both, platelet count fell >20% 12-24 hours after surfacing and moderate (5%) reductions in hemoglobin, red-cell count, and packed-cell volume were observed. Plasma cholesterol and triglyceride levels were depressed postdive as were most plasma enzymes (GOT, GPT, CPK, LDH, ALP). The latter changes were very slight. In the first study, the incidental ingestion of aspirin by some divers did not prevent the loss of platelets even though the platelet-release reaction in response to ADP was inhibited. In the second study the platelet-suppressive drug VK744 was administered, on a double-blind randomized basis, to six divers, six others taking a placebo capsule. Dosage of VK744 was 300 mg TID for 2 days before, 5 days during, 3 days after the saturation dive. The drug inhibited the postdive loss of circulatory platelets and in fact the treated group showed a rebound in platelet count above control values, 48-72 hours postdive. Megathrombocyte counts indicated the production of new platelets in both groups at this point. The treated group also showed a marked and significant reduction in plasma cholesterol and triglycerides, suggesting an antilipidemic effect of the drug. These results confirm previous observations and indicate that postdecompression loss of platelets may be related to sequestering of reactive platelets, possibly by microbubbles, and that the phenomenon can be inhibited by some antiplatelet drugs.

platelets
antiplatelet drugs
saturation diving
blood chemistry
hematology
decompression

The reduction of circulating platelet count in divers decompressed from a hyperbaric environment has been reported by several investigators and was discussed in a recent review in this journal (Philp 1974). It has since been confirmed by other investigators (Valeri, Feingold, Zaroulis, Sphar, and Adams 1974). Information concerning this phenomenon as it relates to saturation diving is, however, scanty. We reported a characteristic fall in circulating platelet counts of seven subjects 24-48 hours following decompression from the HYDRO-LAB Underwater Habitat (Philp, Freeman, Francey, and Ackles 1974). On the basis of animal experiments suggesting that platelets become adherent to, and aggregated by, intravascular bubbles, two experimental platelet-function inhibitors (RA233 and VK744, Pharma Research Canada Ltd.) were administered, on a double-blind basis, to divers before and after they performed a *bounce* decompression dive in a hyperbaric chamber. These drugs are derived from dipyridamole (Persantine, Ciba-Geigy). Both agents significantly reduced

the postdecompression loss of circulating platelets (Philp, Inwood, Ackles, and Radomski 1974). In the present study the effects of two platelet-suppressive agents on platelets and other blood parameters were studied under open-sea saturation diving conditions. In the first instance the drug aspirin was ingested by some divers who were unaware of its potential to interfere with laboratory tests used in the experiment; the study was therefore uncontrolled. In the second case, however, a controlled double-blind investigation of the effects of the experimental agent VK744 was conducted.

METHODS: STUDY I (SITS MISSION)

Subjects

Twenty young-adult divers, 19 male and 1 female, served as the experimental subjects. The divers were university graduate students completing the "Scientist-in-the-Sea" training program, and their instructors. All subjects received a medical examination before diving and after surfacing.

Dive profile

The dives were conducted in the Perry HYDRO-LAB Habitat which is a cylindrical chamber 8 ft in diameter and 16 ft long located in 50 ft of water approximately 1.2 miles off Bell Channel Inlet, Grand Bahama Island. The chamber was maintained at approximately 2.4 ATA (air). The saturation exposure consisted of a 60-hour sojourn in the chamber with frequent swimming excursions occurring within depth limitations of 30-85 ft. Decompression took 13 hours, 37 min including 4 hours, 5 min of 0_2 breathing.[1] Each dive group consisted of three students and one instructor; five groups in all were studied.

Blood collection

Twenty-five ml blood samples were collected from a forearm vein, occluded with a wide tourniquet, into disposable plastic syringes using disposable 20-gauge needles. The blood was immediately distributed into test tubes containing the appropriate anticoagulant for a particular test. Samples were collected the day before the dive, 30 min before the dive, 30 min after decompression, and daily for 3 days thereafter where possible. Although it was not possible to maintain strict fasting conditions at the time of sampling, an effort was made to collect samples just before a meal, so that alimentary lipemia was minimal.

Platelet studies

All glassware contacting whole blood or platelet-rich plasma was silicone-coated. Nine parts of blood were mixed with one part of 3.8% trisodium citrate as the anticoagulant. Platelets were counted visually using a modified Reese-Ecker diluting fluid. Platelet adhesiveness was determined by passing 3 ml of blood through a 5-g column of siliconed glass beads (B. Braun Co.) 0.5 mm in diameter. The percentage of platelets remaining in the columns (% adhesiveness) was calculated from platelet counts done before and after passage through the beads. Platelet aggregation in response to adenosine diphosphate (ADP) in final

[1]Complete details of this dive profile will appear in an article entitled "Hematology and Blood Chemistry in Saturation Diving: II Open-Sea vs. Hyperbaric Chamber" which appears in this issue of *Undersea Biomedical Research.*

concentrations of 7, 14, 28, and 56 x 10^{-7} M was studied at 37°C in a Born aggregometer using platelet-rich plasma obtained by centrifuging citrated whole blood at 750 x *g* for 4 min. Plasma platelet counts were 3-4 x $10^5/mm^3$. Aggregation curve heights were measured in mm at 60s following the addition of the ADP and a dose-response slope was calculated according to the formula

$$\frac{\Sigma xy}{\Sigma x^2} \quad (1)$$

where x = ADP concentration and y = curve height. The numbers thus generated were used to compare the sensitivity of each subject's platelets to ADP-induced aggregation from day to day.

Hematology

Red-cells were counted visually, packed-cell volume was determined by the microhematocrit technique and hemoglobin concentration was measured by the cyamethemoglobin method in an Ames BMI blood analyzer using Hycel reagents.

Blood chemistry

The following determinations were done according to the reagent supplier's specifications in the Ames BMI blood analyzer using the specified manufacturer's reagents: cholesterol (Hycel Laboratories); glutamic oxaloacetic transaminase (GOT) (TransAc, Warner Chilcott); lactic dehydrogenase (LDH) (Lac Dehydstrate, Warner Chilcott); creatine phosphokinase (CPK) (Dade Chemicals); and alkaline phosphatase (ALP) (Hycel Laboratories). Very low-density lipoproteins were measured in a Thorp Nephelometer according to the method of Stone and Thorp (1966). All of the above tests were performed on plasma collected from blood anticoagulated with heparin 40 i.u./ml. Plasma was obtained after centrifuging the whole-blood at 750 x *g* for 15 min.

Statistical analysis

The predive results for each parameter were averaged to yield a control value for each subject to which subsequent postdive values were compared by Fisher's paired *t* test. Because most parameters displayed a wide range of normal values, the postdive changes are expressed as the percentage change from control.

STUDY II (PROTEUS MISSION)

Subjects

Twelve healthy, young-adult males, all members of the University of Western Ontario Diving Club, served as volunteers. All received a thorough medical examination prior to the dive as well as the customary open-water checkout.

Dive profile

The depth, decompression profile, and swimming excursions were as for Study I, except that the dive extended over 5 days, including decompression time.

Blood collection

Blood was collected, as described above, from each diver 48 hours, 24 hours, and immediately predive; once during the dive (dive day 2 or 3); and immediately, 24 hours, 48 hours, and 72 hours postdive. Each indive blood sample was vented with a 20-gauge needle and decompressed in the transport pot from 2.4 ATA. Decompression took at least 10 min. Sample volume was kept to the necessary minimum (20-25 ml) and blood was always collected before a meal to minimize alimentary lipemia.

Platelet studies, hematology, blood chemistry, and statistical analysis were performed as described for Study I. In addition, megathrombocyte indices, i.e. the percentage of platelets having a transverse diameter greater than 2.5 μ, were calculated by counting 1000 platelets per specimen using a Wright-stained blood smear. Glutamic pyruvic transaminase (GPT) (Dade Chemicals) levels in plasma were also measured.

Drug administration

Two members of each saturation team of four divers were assigned randomly to a treatment group or a placebo group. The treatment group received 300 mg three times daily of VK744CL2 (chemical name: 2-[(2-aminoethyl)amino] 4-morpholino-thieno [3,2-d] pyrimidine dihydrochloride) for 24 hours before the dive, during the dive, and for 3 days thereafter. The placebo group received capsules identical in appearance and number but containing lactose. Drug administration was timed so that two blood samples were collected prior to medication and one, i.e. the predive sample, after the subjects had taken four doses (1200 mg). Double-blind procedure was followed throughout and each diver was coded separately so that in the event of a possible drug reaction, his medication could be identified without compromising the entire project. This never occurred. A member of the HYDRO-LAB staff held the drug code.

RESULTS: STUDY I

Classification of subjects

One group of four divers, in addition to their excursion-diving tasks, performed step-test exercise tolerance tests while in the habitat as part of their own physiological study. Since the exercise load was considered to be greater for this dive group than for the others, they were considered separately (exercise group) for statistical comparisons.

In a study involving platelet-function tests, it is customary to admonish subjects against taking salicylate-containing drugs for at least 1 week prior to the experiment. In the present case, because of time and distance, this admonition did not reach the subjects. As data accumulated on ADP-induced platelet aggregation it became apparent that several subjects lacked the second phase of aggregation (i.e. the release of constituents, including ADP, from platelet granules). Since this phenomenon is frequently associated with the ingestion of salicylate-containing drugs, which are capable of inhibiting the release reaction for a week or more after a single dose, all subjects were questioned carefully to elicit and history of recent drug use. The results revealed that five individuals had taken proprietary compounds containing acetylsalicylic acid (aspirin) within the previous few days. Intake ranged from 300 to 900 mg of aspirin taken in one dose, 24 to 96 hours before the collection of the first blood sample. Since these were the subjects whose platelets lacked the release reaction they

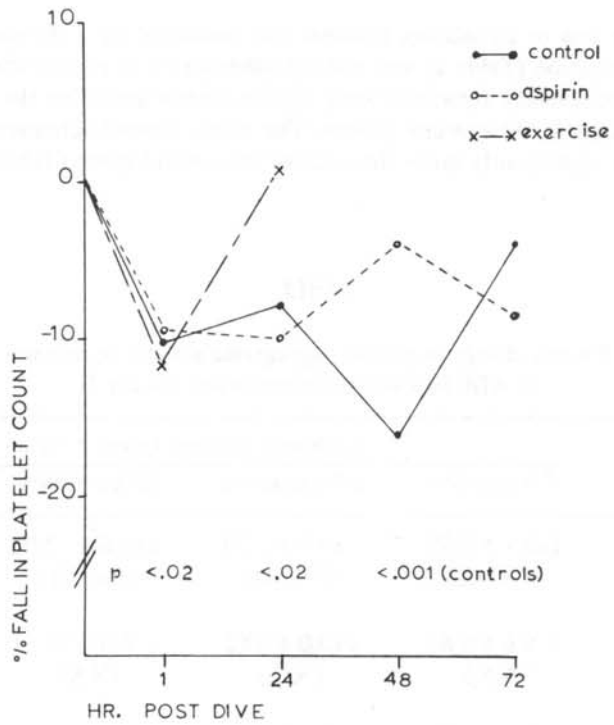

Fig. 1. Study I. The percentage loss of circulating platelets is shown for three groups of divers in Study I. The *control* group (11 subjects) had statistically significant reductions in platelet counts immediately and 24 hours after surfacing. Five divers who had recently taken aspirin (aspirin group) displayed a similar loss of platelets although the difference was not statistically significant. A group of four divers who underwent step-test tolerance tests (exercise group) while in the habitat displayed a transient but nonsignificant loss of platelets immediately after surfacing. Mean, predive platelet counts (± SEM) for each group were: control, 256.7 ± 12.30; aspirin, 230.2 ± 11.11; exercise, 213.5 ± 18.10 ($\times 10^3/mm^3$).

were considered as a separate group (aspirin group) for statistical comparisons. The remaining 11 subjects were identified as the control group. No signs or symptoms of decompression sickness were observed in any subject.

Platelet studies

The percentage changes in circulating platelet counts are illustrated in Fig. 1. There were statistically significant depressions of the circulating platelet count in the control group immediately following decompression and 24 and 48 hours later with a return toward normal values at 72 hours postdecompression. Although the changes in the aspirin group and exercise group were not statistically significant (n = 5 and 4 respectively) on a day-to-day basis, the aspirin group followed the same general pattern as the controls whereas the exercise group showed a return to normal 24 hours after surfacing. This group was not available for further study. Because the platelet count did not fall at the same rate in all subjects, the mean, maximum-percentage fall was calculated for each group. These were: control, 19.0% ± 2.82 SEM ($P < .001$); aspirin, 13.3% ± 5.31 SEM (N.S.) and exercise, 18.2% ± 5.21 SEM (N.S.).

In general, the loss of circulating platelets was paralleled by a decreased sensitivity to ADP-induced aggregation (Table 1) and platelet adhesiveness to glass (Table 2). Again, these differences were statistically significant only for the control group but the same trends were observed in the aspirin and exercise groups. The mean, control adhesiveness value for the exercise group was significantly lower than that of the control group (Table 2).

TABLE 1

Percent change in platelet aggregating activity in response to ADP following decompression (Study I)

Group	*n*	% Change postdive (mean ± SEM)			
		1 h postdive	*24 h postdive*	*48 h postdive*	*72 h postdive*
Control	11	↓10.1 ± 3.59 ($P < .02$)	↓7.7 ± 2.70 ($P < .02$)	↓15.8 ± 2.92 ($P < .02$)	↓6.3 ± 3.07 (N.S.)
Aspirin	5	↓ 9.6 ± 6.83 (N.S.)	↓10.0 ± 4.82 (N.S.)	↓ 4.0 ± 15.51 (N.S.)	↓8.5 ± 5.50 (N.S.)
Exercise	4	↓11.5 ± 6.84 (N.S.)	↓ 0.8 ± 8.23 (N.S.)		

TABLE 2

Percent adhesive platelets, and change in percentage points, following decompression (Study I)

Group	*n*	Predive control value (mean ± SEM)	% Change postdive (mean ± SEM)			
			1 h postdive	*24 h postdive*	*48 h postdive*	*72 h postdive*
Control	11	68.5 ± 2.46	↓6.9 ± 3.53 (N.S.)	↓9.2 ± 3.29 ($P < .02$)	↓7.5 ± 2.45 ($P < .02$)	↓1.5 ± 0.50 (N.S.)
Aspirin	5	57.0 ± 6.02	↓2.8 ± 9.00 (N.S.)	↓2.0 ± 3.56 (N.S.)	↑7.8 ± 4.71 (N.S.)	↑0.5 ± 4.50 (N.S.)
Exercise	4	52.0 ± 6.47	↓13.0 ± 9.17 (N.S.)	↓5.5 ± 15.31 (N.S.)		

Correlation coefficients were calculated between the mean, daily percentage fall in platelet counts for all groups (i.e. all the points in Fig. 1) and the corresponding changes in platelet aggregation (data in Table 1) and in platelet adhesiveness (data in Table 2). The correlation coefficient between the percentage fall in platelet count and percentage fall in aggregating activity was 0.95 whereas that between fall in platelet count and change in platelet adhesiveness was 0.47, suggesting that platelet sensitivity to ADP-induced platelet aggregation is a better indication of platelet viability (assuming that the more active ones are lost from the circulation) than is platelet adhesiveness.

Hematology

Although no statistically significant changes were observed in red-cell count, PCV, or hemoglobin concentrations, values were generally depressed slightly postdive (data not shown).

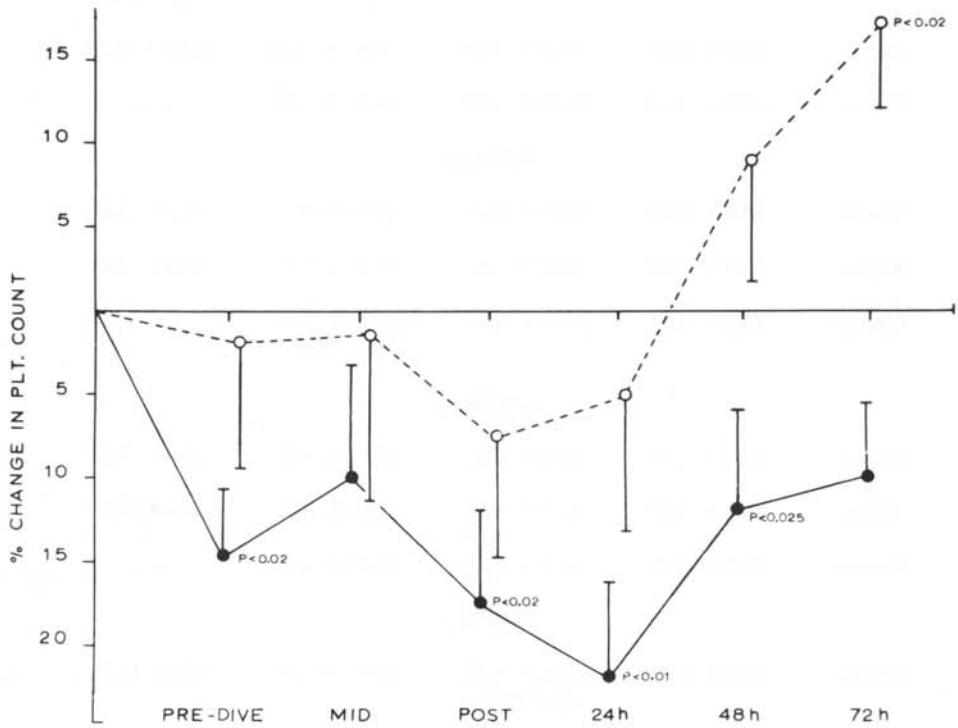

Fig. 2. Study II. The percentage loss of circulating platelets is shown for the placebo (●————●) and treatment (0 – – – 0) groups. *P*-values adjacent to data points indicate significant changes from predive control values. *P*-values at the top of the graph indicate between-group significance at those points (applicable to Figs. 4-6 also). The placebo group manifested statistically significant postdecompression reductions in circulating platelet count which did not occur in the VK744-treated group. Mean predive platelet counts (± SEM) for the two groups were: treatment, 287.5 ± 30.54; placebo 321.8 ± 21.10, ($\times 10^3/mm^3$).

TABLE 3

Blood chemistry (Study I)

Group	Predive values (mean ± SEM)	% Change postdive (mean ± SEM)		
		1 h	*24 h*	*48 h*
		Triglycerides (Mg %)		
Control	75.0 ± 11.08	↓17.1 ± 13.32	↓10.8 ± 13.27	↓6.9 ± 14.72
Aspirin	71.0 ± 15.39	↓21.8 ± 15.20	↓37.1 ± 10.18 ($P < .05$)	↓16.6 ± 18.65
Exercise	88.7 ± 22.99	↑5.9 ± 18.87	↓8.4 ± 18.00	— — —
		Cholesterol (Mg %)		
Control	146.5 ± 7.81	↓2.4 ± 2.35	↓9.6 ± 4.48 ($P < .1$)	↓9.8 ± 4.56 ($P < .1$)
Aspirin	162.6 ± 6.91	↑7.3 ± 4.08	↓6.6 ± 4.56	↓12.4 ± 5.45
Exercise	143.3 ± 7.94	↑10.8 ± 3.64	↑4.5 ± 5.29	— — —
		GOT (i.u.)		
Control	24.9 ± 0.62	↑12.5 ± 5.4	↑3.1 ± 6.5	↑3.3 ± 3.4
Aspirin	27.8 ± 2.30	↑7.2 ± 11.6	↓9.1 ± 5.9	↑0.9 ± 3.4
Exercise	24.3 ± 1.54	↓11.5 ± 3.4	↓21.4 ± 2.0 ($P < .001$)	— — —
		LDH (i.u.)		
Control	55.5 ± 2.59	↓2.2 ± 4.0	↓7.2 ± 4.2	↓0.9 ± 3.2
Aspirin	58.1 ± 3.93	↓3.3 ± 3.8	↓9.5 ± 3.5	↓2.9 ± 10.1
Exercise	61.9 ± 7.48	↓4.2 ± 2.6	↑1.15 ± 0.91	— — —
		CPK (i.u.)		
Control	30.3 ± 2.31	↓46.0 ± 9.36 ($P < .001$)	↓22.7 ± 15.97	↓38.0 ± 15.36
Aspirin	32.4 ± 3.25	↓45.9 ± 6.68 ($P < .001$)	↓44.2 ± 9.46 ($P < .01$)	↓63.7 ± 13.10 ($P < .01$)
Exercise	17.2 ± 4.03	↓39.8 ± 20.86	↓3.5 ± 35.20	— — —
		Alkaline Phosphatase (i.u.)		
Exercise	16.1 ± 4.34	↓15.0 ± 5.1 ($P = .05$)	↑3.4 ± 7.50	— — —

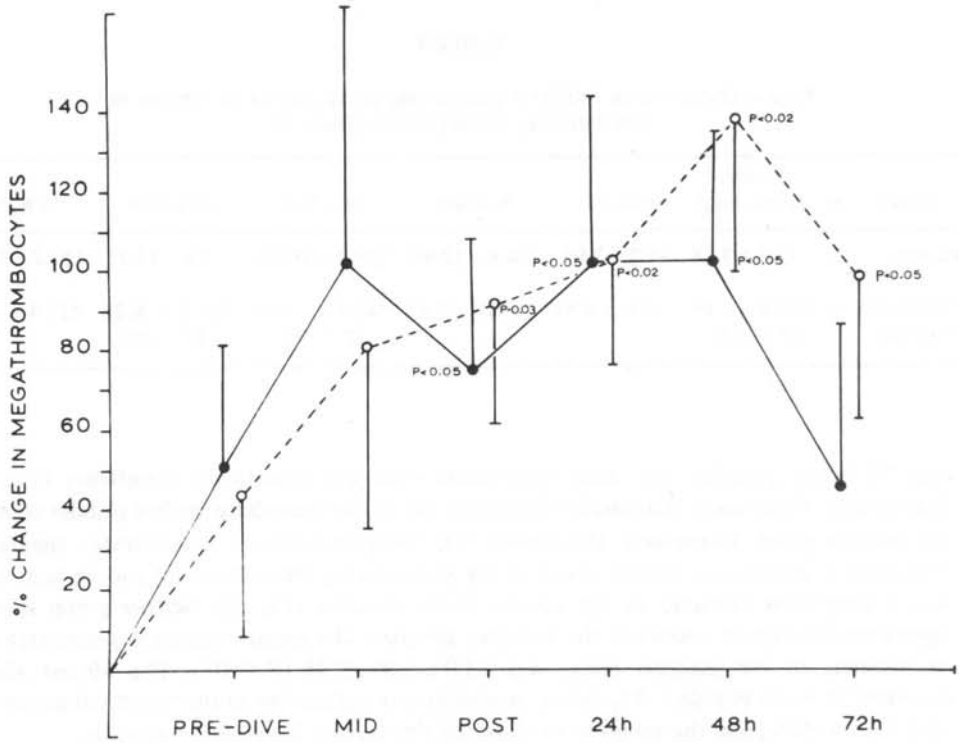

Fig. 3. Study II. Megathrombocyte counts (% platelets > 2.5 μ diameter) were roughly doubled in both groups in most postdive samples; the VK744-treated group (0 ––– 0) showing a greater elevation 48 and 72 hours postdive than the placebo group (●————●). Mean previde values (± SEM) for both groups were respectively, 8.9 ± 0.68% and 11.0 ± 1.73%.

Blood chemistry (Table 3)

Lipid values were generally depressed in the postdive samples of the control and aspirin groups. The depression was statistically significant only for the 24-hour postdive triglycerides in the aspirin group although depression of cholesterol levels approached significance in the control group at 24 and 48 hours postdive. No statistically significant changes were observed in the exercise group.

Plasma enzyme levels (GOT, LDH, CPK, ALP) either did not change significantly or were slightly depressed in most of the postdecompression samples in all three groups (Table 3). With respect to GOT and LDH, the level of depression was very slight although in some cases statistically significant. Depressions of CPK values were, however, frequently statistically significant.

RESULTS: STUDY II

Platelets

No signs or symptoms of decompression sickness occurred in either group. The control (placebo) group manifested a progressive loss of circulating platelets which was statistically significant immediately postdive and 24 hours postdive. Values were still below normal at 48

TABLE 4

Percent change (mean ± SEM) in platelet aggregating activity in response to ADP following decompression (Study II)

Group	*n*	Predive (after drug)	Mid-dive	Postdive	24 h Post	48 h Post	72 h Post
Placebo	6	↓2.6 ± 9.18	↓1.7 ± 7.04	↑14.4 ± 12.40	↑10.2 ± 10.00	↑1.5 ± 12.75	↑12.2 ± 12.81
Treatment (VK744)	6	↑20.0 ± 5.10 ($P < .02$)	↓2.5 ± 5.81	↑8.0 ± 9.45	↑20.4 ± 7.19 ($P < .05$)	↑14.2 ± 6.21 ($P < .05$)	↑11.4 ± 7.61

and 72 hours postdive but these differences were not statistically significant (Fig. 2). Surprisingly, there was a statistically significant fall in the immediate predive platelet count in the placebo group. Conversely, the treated (VK744) group did not demonstrate a significant reduction in circulating platelet count at any point during the experiment and, in fact, there was a significant rebound in the counts 72 hr postdive (Fig. 2). Neither group showed significant changes in counts in the mid-dive samples. The mean, maximum-percentage loss of platelets in the placebo group was 23.0 ± 5.09 SEM ($P < .01$). This almost always occurred 24 hours postdive. The mean, maximum-percentage loss in the treatment group was 15.0 ± 6.44 (N.S.) and the values were scattered throughout the postdive samples.

Megathrombocyte counts (Fig. 3) were significantly increased in both groups in the postdive samples, with the increase being more pronounced in the VK744-treated group at a time (48-72 hours) coinciding with the elevation in platelet count above control values. Megathrombocyte counts showed a moderate but not statistically significant increase in the mid-dive samples of both groups.

The treated group showed statistically significant increases in platelet sensitivity to ADP-induced aggregation in the immediate predive sample and again at 24 and 48 hours postdive (Table 4). No statistically significant changes were observed in the placebo group although there was a trend toward increased activity in the postdive samples (Table 4).

Platelet adhesiveness was significantly decreased in the treated group immediately predive (i.e. after 24 hours of medication) and significantly increased in both groups in the mid-dive samples (Table 5).

TABLE 5

Percent adhesive platelets, and change in percentage points, following decompression (Study II)

Group	*n*	Control adhesiveness (mean ± SEM)	Predive (after drug)	% Change (mean ± SEM)				
				Mid-dive	*Postdive*	*24 h post*	*48 h post*	*72 h post*
Placebo	6	47.5 ± 5.40	↓16.5 ± 9.26	↑36.2 ± 16.07 ($P = .05$)	↑20.8 ± 14.91	↓1.7 ± 12.45	↓17.2 ± 16.02	↓4.3 ± 6.07
Treatment (VK 744)	6	47.3 ± 2.72	↓40.5 ± 11.33 ($P < .02$)	↑33.7 ± 12.10 ($P < .05$)	↓20.0 ± 13.55	↓15.2 ± 8.93	↑9.7 ± 10.30	↑4.2 ± 16.68

Hematology

Changes in PCV are shown in Fig. 4 and similar changes were reflected in hemoglobin concentrations (not shown). Both the treatment group and the placebo group displayed slight, progressive, postdive reductions in packed cell volume and hemoglobin concentration amounting to approximately 5% loss in the 48- and 72-hour samples, the differences being statistically significant. Neither group showed statistically significant changes in red-cell counts.

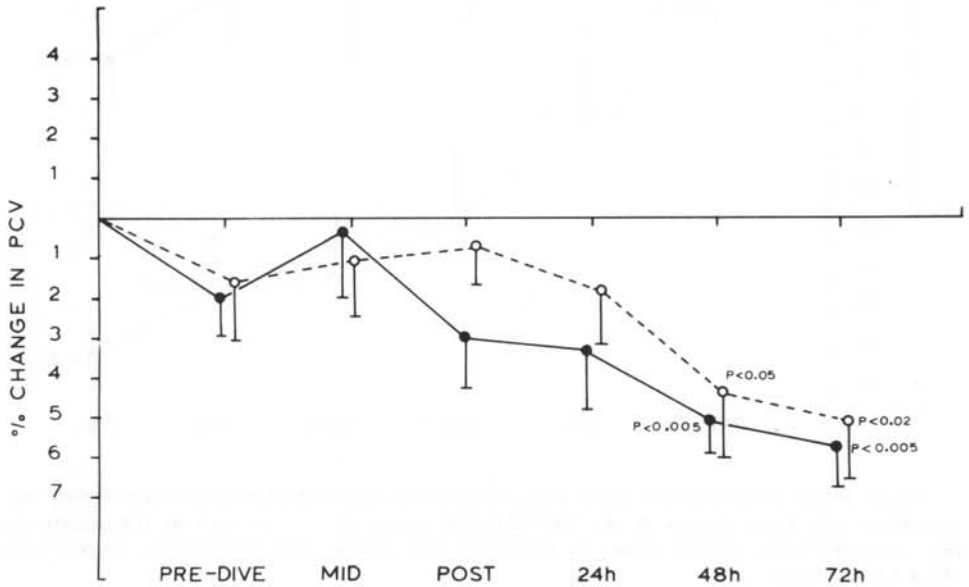

Fig. 4. Study II. Packed-cell volume for both treatment (0 – – – 0) and placebo (●————●) groups showed a slight but statistically significant depression 48 and 72 hours postdive. The mean predive values (± SEM) for the two groups were respectively, 46.2 ± 0.40 and 47.2 ± 0.95.

Blood chemistry

Both plasma cholesterol and triglyceride levels decreased throughout the dive and during the postdive observation period in both treatment and placebo groups (Figs. 5 and 6). The reduction in plasma lipid levels was more pronounced and statistically significant in the treatment group. When VK744 was added to plasma in vitro there was no interference with chemical (cholesterol) or enzymatic procedures, indicating that any reductions of lipid and enzyme activity in the drug group compared to the placebo group was real.

LDH levels tended to be slightly (< 20%) elevated postdive, reaching statistical significance ($P < .05$) postdive and 48 to 72 hours postdive in both groups. CPK was significantly ($P < .05$) increased (250%) in the predive sample of the placebo group. No other noteworthy changes occurred in CPK or transaminase (GOT, GPT) levels. After the dive, ALP showed a slight (12-20%) but significant ($P < .05$) depression in the mid- and postdive samples of the treatment group only. These results are summarized in Table 6.

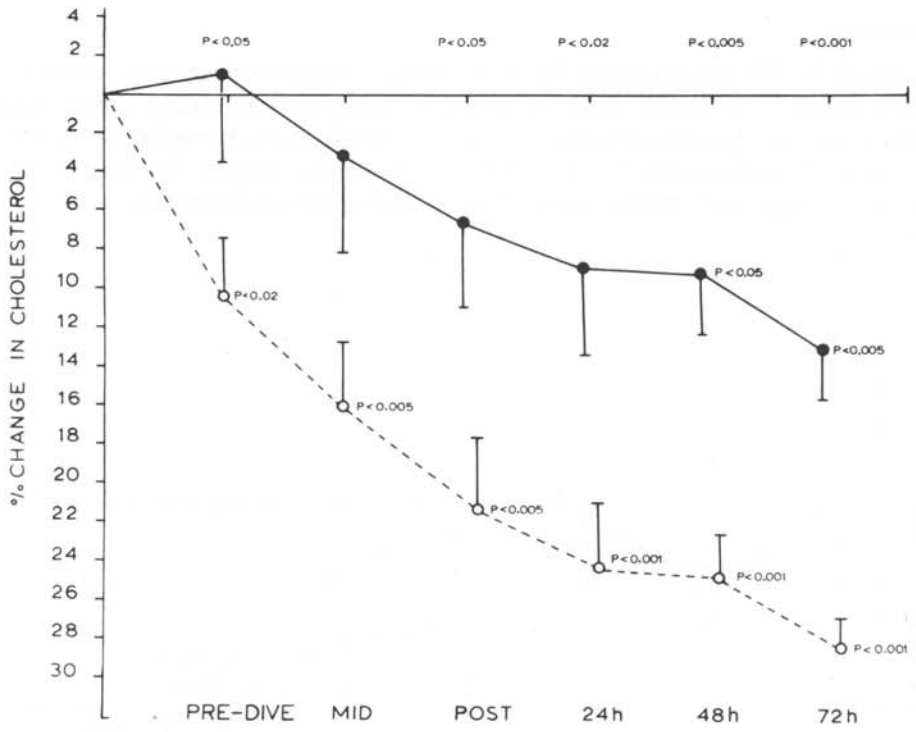

Fig. 5. Study II. Cholesterol levels were significantly depressed in both groups post-dive but the depression was much greater in the VK744-treated group (0 --- 0) than in the placebo group (●——●). Mean predive values (± SEM) for both groups were respectively, 181.9 ± 7.89 and 187.4 ± 8.46 (mg %).

DISCUSSION

Platelet studies

The progressive, postdive reduction in circulating platelet count has been confirmed in two separate saturation dives in the HYDRO-LAB habitat, one totaling 3 days and the other 5 days duration (decompression time included). In both instances the reduction averaged 15-35% and occurred usually within 24 hours of surfacing with no signs or symptoms of decompression sickness. This loss was not as great as previously reported (Philp, Freeman et al. 1974) in six divers following a similar dive profile in the same habitat; however, the latter group contained two individuals who lost in excess of 50% of the circulating platelet count. Surprisingly, the control group in the second study (Proteus) showed a statistically significant reduction in platelet count in the predive sample. Since the data was accumulated from three separate dives and spanned a period of 15 days, the loss cannot likely be attributed to experimental artifact. One possible explanation relates to the fact that these subjects were all amateur scuba divers who had never before experienced a saturation dive. An understandable degree of excitement or apprehension could have resulted in excessive catecholamine output with an increase in platelet adhesiveness and subsequent sequestering

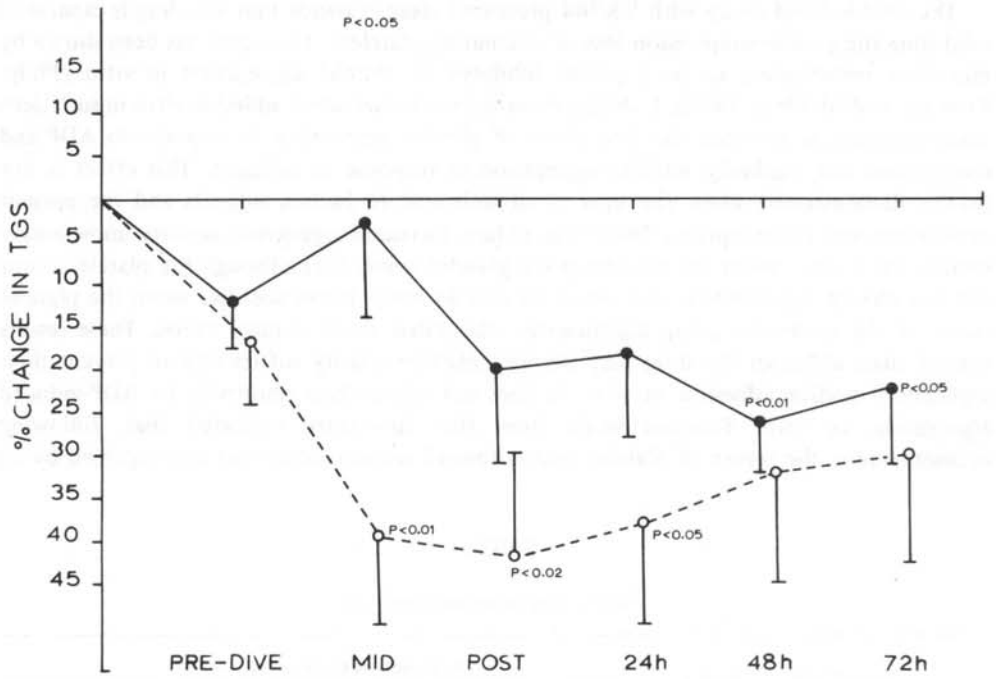

Fig. 6. Study II. Triglyceride levels followed the same pattern as for cholesterol (Fig. 5). The VK744-treated group (0 – – – 0) showed a much more pronounced lowering of triglycerides than did the placebo (●————●) group. Mean predive values for both groups were, respectively, 86.8 ± 13.06 and 116.4 ± 23.22 (mg %).

of platelets in the microcirculation. Samples collected on the third saturation day in Study II did not demonstrate a significant change in the platelet counts, suggesting that the loss of platelets is related to decompression rather than to the hyperbaric environment itself. In Study I (SITS) both parameters were reduced in parallel with the reduction in platelet count with a high degree of correlation being noted between loss of platelets and loss of aggregating activity. In the second study these trends were not as clear-cut. The control group did demonstrate a significant reduction in platelet adhesiveness coinciding with the predive reduction in platelet count. Adhesiveness was markedly increased in the midsaturation dive sample, but because of the logistic problems of transporting samples from an undersea hyperbaric environment to an on-shore laboratory, interpretation is difficult.

With respect to the incidental ingestion of aspirin which occurred in Study I, it can be said only that the prevention of the release reaction (second phase of ADP-induced aggregation) by the recent ingestion of this drug does not itself assure the prevention of a loss of circulating platelets. The smallness of the group and the lack of controlled dosage and time of ingestion prevent firmer conclusions. It is conceivable that higher doses or more frequent administration might have prevented platelet loss. It is interesting to note, however, that animal experiments have failed to demonstrate such an effect (Inwood 1973) and aspirin has not been shown to reduce the incidence or severity of experimental decompression sickness (Adler 1964; Williams, Lyons, Bridge, and Cook 1946; Bennett and Brock 1969).

The double-blind study with VK744 presented clear evidence that the drug is capable of inhibiting the postdecompression loss of circulating platelets. This agent has been shown by numerous investigators to be a potent inhibitor of platelet aggregation in vitro (Philp, Francey, and McElroy 1973). It differs from aspirin in that when added in vitro in sufficient concentrations, it prevents the first phase of platelet aggregation in response to ADP and epinephrine and markedly inhibits aggregation in response to collagen. This effect is not readily demonstrated when the drug is administered to human subjects and the present experiment was no exception. There was, in fact, increased aggregating activity immediately predive (at a time when the placebo-group platelet count fell) although the platelet count did not change significantly, and again 24 and 48 hours hours postdive when the platelet count of the treatment group significantly rebounded above control values. These results suggest that, although the drug may suppress platelet activity sufficiently to prevent their aggregation and/or adhesion in vivo, it does not affect their sensitivity to ADP-induced aggregation ex vivo. Previous work from this laboratory indicated that, following decompression, the return of platelet counts toward normal values was accompanied by an

TABLE 6

Plasma enzyme levels (Study II)

Group	Control value (mean ± SEM)	Predive (after drug)	% Change from Control (Mean ± SEM)				
			Mid-dive	*Postdive*	*24 h post*	*48 h post*	*72 h post*
			GOT (i.u.)				
Placebo	29.0 ± 1.91	↑4.1 ± 2.78	↑7.2 ± 8.32	↑6.7 ± 7.73	↓2.9 ± 6.24	↓2.6 ± 4.85	↓5.9 ± 3.97
Treatment (VK744)	34.0 ± 3.39	↓4.2 ± 4.25	↓6.3 ± 5.48	↓6.9 ± 9.43	↓12.7 ± 8.81	↓11.8 ± 7.74	↓14.3 ± 6.50
			GPT (i.u.)				
Placebo	13.7 ± 1.80	↑20.7 ± 20.50	↓2.8 ± 10.93	↑9.6 ± 13.16	↓21.9 ± 5.33 ($P < .01$)	↓14.1 ± 14.47	↓2.3 ± 10.89
Treatment (VK744)	18.5 ± 4.57	↓3.5 ± 10.63	↓17.5 ± 13.02	↓16.7 ± 8.98	↓38.1 ± 7.60 ($P < .005$)	↓10.6 ± 12.26	↓27.7 ± 11.60
			LDH (i.u.)				
Placebo	55.2 ± 3.41	↑2.5 ± 3.81	↑13.2 ± 7.13	↑16.6 ± 5.28 ($P < .05$)	↑6.5 ± 7.13	↑9.9 ± 3.75 ($P < .05$)	↑11.6 ± 6.52
Treatment (VK744)	57.1 ± 4.01	↑0.1 ± 2.21	↓0.5 ± 5.40	↑12.5 ± 2.94 ($P < .01$)	↑13.2 ± 8.95	↑11.8 ± 5.72	↑15.0 ± 5.45 ($P < .05$)
			CPK (i.u.)				
Placebo	6.2 ± 2.76	↑245.9 ± 77.77 ($P < .03$)	↑98.5 ± 58.77	↑88.2 ± 90.20	↑90.8 ± 107.96	↑64.7 ± 76.47	↓27.5 ± 45.3
Treatment (VK744)	4.4 ± 1.84	↑105.0 ± 60.22	↑90.0 ± 63.65	↑72.2 ± 54.42	↑117.7 ± 51.38	↑78.4 ± 54.29	↑125.5 ± 47.58 ($P < .05$)
			ALKALINE PHOSPHATASE				
Placebo	12.0 ± 1.13	↑2.0 ± 3.51	↓2.3 ± 8.12	↑5.8 ± 3.00	↑2.7 ± 2.52	↑1.6 ± 3.33	↑5.9 ± 3.63
Treatment (VK744)	15.2 ± 1.35	↓6.7 ± 4.09	↓17.7 ± 4.21 ($P < .01$)	↓11.7 ± 4.53 ($P < .05$)	↓7.5 ± 3.24	↓8.2 ± 4.19	↓6.6 ± 4.24

increase in large platelets (megathrombocytes), which are believed to be newly formed from megakaryocytes in bone marrow (Philp, Ackles, Inwood, Livingston, Achimastos, Binns-Smith, and Radomski 1972; Philp, Inwood et al. 1974). In the present instance, the rebound in platelet counts may be due to such formation in bone marrow, even though no apparent loss of circulating platelets occurred. The increase in megathrombocyte count which accompanied the rebound would tend to support this. Three possible explanations suggest themselves.

1) The release of new platelets into the circulation is not connected with the reduction in circulating count; this is not in accord with current theories of thrombopoiesis (Albigaard and Simone 1967).

2) The slight reduction in platelet count was sufficient to stimulate thrombopoiesis. It seems unlikely that a 5% (not statistically significant) reduction in count could initiate a 20% increase (statistically significant).

3) The event that normally leads to a loss of circulating platelets could, in the presence of the inhibitor drug VK744, initiate the release of unidentified platelet constituents which participate in a feedback mechanism to stimulate the production of new platelets, without damaging platelets sufficiently to cause their removal from the circulation. Animal experiments (Philp 1974) suggest that this event could be the presence of intravascular bubbles. The increased sensitivity to ADP-induced aggregation which accompanied the rebound in count is again suggestive of the release of new platelets since young platelets are more reactive in this test (Karpatkin 1972). Valeri et al. (1974) presented evidence suggesting that a correlation existed between the reduced circulating platelet count following decompression and decreased platelet production postdive. However, the fact that VK744, an antiaggregating agent, prevents loss of platelets does not support such a hypothesis; rather it supports the contention that platelets are destroyed postdecompression (Philp et al. 1972; Warren, Philp and Inwood 1973). Moreover, the megathrombocyte counts indicated increased platelet production when the counts were depressed. This would suggest that megakaryopoiesis was not impaired and, moreover, the loss of platelets may have been greater than the counts would indicate.

Hematology

In both studies there was evidence of a mild, progressive anemia following decompression, thus confirming previous observations of this laboratory (Philp, Freeman et al. 1974) and other authors (Widell, Pilmanis, Chapman, Pilmanis, and Given 1973) following saturation dives in the HYDRO-LAB habitat. Several theories have been postulated to explain this phenomenon including hemodilution, suppression of red-cell formation by hyperoxia, and hemorrhagic anemia resulting from repeated blood sampling (see Philp, 1974 for review). Philp (1974), on reviewing the literature, found that this *anemic* response appeared most often in open-sea saturation dives and speculated that it might be due to redistribution of body fluids in response to intermittent periods of relative weightlessness. At the moment, no fully adequate explanation exists for this phenomenon.

Blood chemistry

Although statistically significant changes in the various plasma enzymes were noted, none of these was of physiological significance. In brief, it can be stated that neither the conditions of the saturation dive nor the administration of the drug VK744 under hyperbaric conditions caused detectable damage to liver or other tissues. In Study I there

was evidence of a slight, progressive decline in the postdive levels of most enzymes measured. This was reported previously under similar experimental conditions (Philp, Freeman et al. 1974). In Study II, both placebo and treatment groups had slight (12-18%) but statistically significant elevations of LDH levels postdive. Again these were not physiologically significant and may be related to the fact that many of the divers suffered brief bouts of gastroenteritis which was currently epidemic on Grand Bahama Island.

Plasma cholesterol and triglyceride levels tended to be slightly but significantly depressed in both studies. Animal experiments have shown that plasma lipids tend to accumulate, with platelets, at blood-bubble interfaces (Philp, Inwood, and Warren, 1972; Warren et al. 1973). The slight loss of plasma lipids, together with the loss of platelets, may be indicative of a degree of bubble formation during decompression. However, no symptoms of decompression sickness were reported. Most noteworthy was the marked and significant loss of lipids in the treatment group which may indicate some antilipemic effect for this drug. The significant reduction in ALP in the treatment group is probably associated with the reduction in triglycerides since this enzyme is partially bound to lipoprotein (Lawrence and Melnick 1961).

In summary, the postdive reduction in circulating platelet count following saturation diving has been confirmed. This loss of platelets was prevented by the experimental platelet suppressive drug VK744 but not by the recent, incidental ingestion of aspirin even though the platelet release reaction was inhibited. Plasma enzyme levels did not rise significantly, indicating that no tissue damage was induced either by the hyperbaric environment by decompression or by the drug. VK744 also manifested an antilipemic activity.

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Philp, R. B., D. Freeman, I. Francey, and B. Bishop. 1975. Paramètres hématologiques et biochimie sanguine au cours des plongées à saturation: I. Les substances antiplaquettaires l'aspirine et VK744. Undersea Biomed. Res. 2(4):233-249.–La chimie sanguine et des paramètres cellulaires ont été étudiés avant, au cours de, et après deux plongées à saturation (2.4 ATA) dans le habitat du HYDRO-LAB. Chaque fois, une baisse de la numération plaquettaire de 20 p. c., survenue 12-24 h après le retour au surface, et des diminutions modérées (5 p.c.) de l'hémoglobine, de la numération érythrocytaire, et du volume des érythrocytes centrifugés ont été observées. Les taux plasmatiques du cholestérol et des triglycérides, comme ceux des enzymes plasmatiques (GOT, GPT, CPK, LDH, ALP) restaient diminués après la plongée; les altérations enzymatiques étaient peu importantes. Au cours de la première étude, l'ingestion de l'aspirine par quelques plongeurs n'a pas empêché la perte plaquettaire, quoique la résponse plaquettaire à l'ADP se soit trouvée inhibée. Dans la seconde étude, le médicament antiplaquettaire VK744 a été administré, à double insu, à six plongeurs; six autres recevaient un placébo. Les plongeurs prenaient le VK744 (300 mg trois fois par jour) pendant 2 jours avant la plongée, 5 jours au cours de la plongée, et 3 jours après la plongée. Le médicament a empêché la perte post-plongée des plaquettes circulatoires, et chez les plongeurs

traités on a même observé une augmentation des plaquettes au delà des valeurs témoins 48-72 h aprés la plongée. Les taux de mégathrombocytes ont traduit la production de nouveaux plaquettes des deux groupes à ce moment. Chez les plongeurs traités on a aussi constaté une réduction importante de significative des taux plasmatiques du cholestérol et des triglycérides, ce qui suggère un effect antilipémique de la substance. Ces résultats confirment les observations précédentes, et suggèrent que la perte post-décompression des plaquettes peut-être due à une séquestration des plaquettes réactives, peut-être par des microbulles, et que ce phénomène peut être inhibé par quelques-uns des médicaments antiplaquettaires.

plaquettes sanguines
médicaments antiplaquettaires
plongée à saturation
chimie sanguine
hématologie
décompression

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